AROMATIC SULFONE HYDROXAMIC ACIDS
AND THEIR USE AS PROTEASE INHIBITORS

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

5   **[1]**   This patent claims priority as a continuation-in-part to U.S. Patent Application Serial No. 09/570,731 (filed May 12, 2000), which, in turn, claims priority to U.S. Patent Application Serial Nos. 09/311,837 (filed May 14, 1999) and 09/256,948 (filed February 24, 1999), which, in turn, claim priority to U.S. Patent Application Serial Nos. 09/191,129 (filed November 13, 1998), 09/186,410

10   (filed November 5, 1998), 60/066,007 (filed November 14, 1997), 60/095,347 (filed August 4, 1998), 60/095,501 (filed August 6, 1998), and 60/101,080 (filed September 18, 1998). The entire texts of the above-referenced patent applications are incorporated by reference into this patent.

15   ## FIELD OF THE INVENTION

     [2]   This invention is directed generally to proteinase (also known as "protease") inhibitors, and, more particularly, to aromatic sulfone hydroxamate compounds (also known as "aromatic sulfone hydroxamic acid compounds") and salts thereof (particularly pharmaceutically acceptable salts) that, *inter alia,* inhibit

20   matrix metalloproteinase (also known as "matrix metalloprotease" or "MMP") and/or aggrecanase activity. This invention also is directed to pharmaceutical compositions of such compounds and salts, and methods of using such compounds and salts to prevent or treat conditions associated with MMP and/or aggrecanase activity, particularly pathological conditions.

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BACKGROUND OF THE INVENTION

     [3]   Connective tissue is a required component of all mammals. It provides rigidity, differentiation, attachments, and, in some cases, elasticity. Connective tissue components include, for example, collagen, elastin,

proteoglycans, fibronectin, and laminin. These biochemicals make up (or are components of) structures, such as skin, bone, teeth, tendon, cartilage, basement membrane, blood vessels, cornea, and vitreous humor.

[4] Under normal conditions, connective tissue turnover and/or repair processes are in equilibrium with connective tissue production. Degradation of connective tissue is carried out by the action of proteinases released from resident tissue cells and/or invading inflammatory or tumor cells.

[5] Matrix metalloproteinases, a family of zinc-dependent proteinases, make up a major class of enzymes involved in degrading connective tissue. Matrix metalloproteinases are divided into classes, with some members having several different names in common use. Examples are: MMP-1 (also known as collagenase 1, fibroblast collagenase, or EC 3.4.24.3); MMP-2 (also known as gelatinase A, 72kDa gelatinase, basement membrane collagenase, or EC 3.4.24.24), MMP-3 (also known as stromelysin 1 or EC 3.4.24.17), proteoglycanase, MMP-7 (also known as matrilysin), MMP-8 (also known as collagenase II, neutrophil collagenase, or EC 3.4.24.34), MMP-9 (also known as gelatinase B, 92kDa gelatinase, or EC 3.4.24.35), MMP-10 (also known as stromelysin 2 or EC 3.4.24.22), MMP-1 I (also known as stromelysin 3), MMP-12 (also known as metalloelastase, human macrophage elastase or HME), MMP- 13 (also known as collagenase 111), and MMP- 14 (also known as MT1-MMP or membrane MMP). *See, generally*, Woessner, J.F., "The Matrix Metalloprotease Family" in *Matrix Metalloproteinases*, pp.1-14 (Edited by Parks, W.C. & Mecham, R.P., Academic Press, San Diego, CA 1998).

[6] Excessive breakdown of connective tissue by MMPs is a feature of many pathological conditions. Inhibition of MMPs therefore provides a control mechanism for tissue decomposition to prevent and/or treat these pathological conditions. Such pathological conditions generally include, for example, tissue destruction, fibrotic diseases, pathological matrix weakening, defective injury repair, cardiovascular diseases, pulmonary diseases, kidney diseases, liver diseases,

and diseases of the central nervous system. Specific examples of such conditions include, for example, rheumatoid arthritis, osteoarthritis, septic arthritis, multiple sclerosis, a decubitis ulcer, corneal ulceration, epidermal ulceration, gastric ulceration, tumor metastasis, tumor invasion, tumor angiogenesis, periodontal

5   disease, liver cirrhosis, fibrotic lung disease, emphysema, otosclerosis, atherosclerosis, proteinuria, coronary thrombosis, dilated cardiomyopathy, congestive heart failure, aortic aneurysm, epidermolysis bullosa, bone disease, Alzheimer's disease, and defective injury repair *(e.g.,* weak repairs, adhesions such as post-surgical adhesions, and scarring).

10   [7]   Matrix metalloproteinases also are involved in the biosynthesis of tumor necrosis factors (TNFs). Tumor necrosis factors are implicated in many pathological conditions. TNF-α, for example, is a cytokine that is presently thought to be produced initially as a 28 kD cell-associated molecule. It is released as an active, 17 kD form that can mediate a large number of deleterious effects *in vitro*

15   and *in* vivo. TNF-α can cause and/or contribute to the effects of inflammation *(e.g.,* rheumatoid arthritis), autoimmune disease, graft rejection, multiple sclerosis, fibrotic diseases, cancer, infectious diseases *(e.g.,* malaria, mycobacterial infection, meningitis, etc.), fever, psoriasis, cardiovascular diseases *(e.g.,* post-ischemic reperfusion injury and congestive heart failure), pulmonary diseases, hemorrhage,

20   coagulation, hyperoxic alveolar injury, radiation damage, and acute phase responses like those seen with infections and sepsis and during shock *(e.g.,* septic shock and hemodynamic shock). Chronic release of active TNF-α can cause cachexia and anorexia. TNF-α also can be lethal.

[8]   Inhibiting TNF (and related compounds) production and action is an

25   important clinical disease treatment. Matrix metalloproteinase inhibition is one mechanism that can be used. MMP *(e.g.,* collagenase, stromelysin, and gelatinase) inhibitors, for example, have been reported to inhibit TNF-α release. *See, e.g.,* Gearing et al. *Nature* 376, 555-557 (1994). *See also,* McGeehan et al. *See also, Nature* 376, 558-561 (1994). MMP inhibitors also have been reported to inhibit

TNF-α convertase, a metalloproteinase involved in forming active TNF-α. *See, e.g.,* WIPO Int'l Pub. No. WO 94/24140. *See also,* WIPO Int'l Pub. No. WO 94/02466. *See also,* WIPO Int'l Pub. No. WO 97/20824.

[9]   Matrix metalloproteinases also are involved in other biochemical processes in mammals. These include control of ovulation, post-partum uterine involution, possibly implantation, cleavage of APP (β-amyloid precursor protein) to the ainyloid plaque, and inactivation of (α_I-protease inhibitor (α_1 -PI). Inhibiting MMPs therefore may be a mechanism that may be used to control of fertility. In addition, increasing and maintaining the levels of an endogenous or administered serine protease inhibitor *(e.g.,* α_1 -PI) supports the treatment and prevention of pathological conditions such as emphysema, pulmonary diseases, inflammatory diseases, and diseases of aging *(e.g.,* loss of skin or organ stretch and resiliency).

[10]   Numerous metalloproteinase inhibitors are known. *See, generally,* Brown, P.D., "Synthetic Inhibitors of Matrix Metalloproteinases," in *Matrix Metalloproteinases,* pp. 243-61 (Edited by Parks, W.C. & Mecham, R.P., Academic Press, San Diego, CA 1998).

[11]   Metalloproteinase inhibitors include, for example, natural biochemicals, such as tissue inhibitor of metalloproteinase (TIMP), $\alpha2$-macroglobulin, and their analogs and derivatives. These are high-molecular-weight protein molecules that form inactive complexes with metalloproteinases.

[12]   A number of smaller peptide-like compounds also have been reported to inhibit metalloproteinases. Mercaptoamide peptidyl derivatives, for example, have been reported to inhibit angiotensin coniierting enzyme (also known as ACE) *in vitro* and *in vivo.* ACE aids in the production of angiotensin II, a potent pressor substance in mammals. Inhibiting ACE leads to lowering of blood pressure.

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Attorney Docket 3124/9A/US
HDP Docket 6794-000017/DVE

[13]   A wide variety of thiol compounds have been reported to inhibit MMPs. *See, e.g.,* WO95/12389. *See also,* WO96/11209. *See also,* U.S. Patent No. 4,595,700. *See also,* U.S. Patent No. 6.013,649.

[14]   A wide variety of hydroxamate compounds also have been reported to inhibit MMPs. Such compounds reportedly include hydroxamates having a carbon backbone. *See, e.g.,* WIPO Int'l Pub. No. *WO* 95/29892. *See also,* WIPO Int'l Pub. No. WO 97/24117. *See also,* WIPO Int'l Pub. No. WO 97/49679. *See also,* European Patent No. EP 0 780 386. Such compounds also reportedly include hydroxamates having peptidyl backbones or peptidomimetic backbones. *See, e.g,* WIPO Int'l Pub. No. WO 90/05719. *See also,* WIPO Int'l Pub. No. WO 93/20047. *See also,* WIPO Int'l Pub. No. WO 95/09841. *See also,* WIPO Int'l Pub. No. WO 96/06074. *See also,* Schwartz et al., Progr. *Med. Chem.,* 29:271-334(1992). *See also,* Rasmussen et al., *PharmacoL Ther.,* 75(1): 69-75 (1997). *See also,* Denis et al., *Invest New Drugs,* 15(3): 175-185 (1997). Sulfamato hydroxamates have additionally been reported to inhibit MMPs. *See,* WIPO Int'l Pub. No. WO 00/46221. And various aromatic sulfone hydroxamates have been reported to inhibit MMPs. *See,* WIPO Int'l Pub. No. WO 99/25687. *See also,* WIPO Int'l Pub. No. WO 00/50396. *See also,* WIPO Int'l Pub. No. WO 00/69821.

[15]   It is often advantageous for an MMP inhibitor drug to target a certain MMP(s) over another MMP(s). For example, it is typically preferred to inhibit MMP-2, MMP-3, MMP-9, and/or MMP-13 (particularly MMP-13) when treating and/or preventing cancer, inhibiting of metastasis, and inhibiting angiogenesis. It also is typically preferred to inhibit MMP-13 when preventing and/or treating osteoarthritis. *See, e.g.,* Mitchell et al., J *Clin. Invest.,* 97:761-768 (1996). *See also,* Reboul et al., *J Clin. Invest.,* 97:2011-2019 (1996). Normally, however, it is preferred to use a drug that has little or no inhibitory effect on MMP-1 and MMP-14. This preference stems from the fact that both MMP-1 and MMP-14 are involved in several homeostatic processes, and inhibition of MMP-1 and/or MMP-14 consequently tends to interfere with such processes.

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[16] Many known MMP inhibitors exhibit the same or similar inhibitory effects against each of the MMPs. For example, batimastat (a peptidomimetic hydroxamate) has been reported to exhibit IC_{50} values of from about 1 to about 20 nM against each of MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9. Marimastat (another peptidomimetic hydroxamate) has been reported to be another broad-spectrum MMP inhibitor with an enzyme inhibitory spectrum similar to batimastat, except that Marimastat reportedly exhibited an IC_{50} value against MMP-3 of 230 nM. *See* Rasmussen et al., *Pharmacol. Ther.*, 75(1): 69-75 (1997).

[17] Meta analysis of data from Phase I/II studies using Marimastat in patients with advanced, rapidly progressive, treatment-refractory solid tumor cancers (colorectal, pancreatic, ovarian, and prostate) indicated a dose-related reduction in the rise of cancer-specific antigens used as surrogate markers for biological activity. Although Marimastat exhibited some measure of efficacy via these markers, toxic side effects reportedly were observed. The most common drug-related toxicity of Marimastat in those clinical trials was musculoskeletal pain and stiffness, often commencing in the small joints in the hands, and then spreading to the arms and shoulder. A short dosing holiday of 1-3 weeks followed by dosage reduction reportedly permits treatment to continue. *See* Rasmussen et al., *Pharmacol. Ther.*, 75(1): 69-75 (1997). It is thought that the lack of specificity of inhibitory effect among the MMPs may be the cause of that effect.

[18] Another enzyme implicated in pathological conditions associated with excessive degradation of connective tissue is aggrecanase, particularly aggrecanase-1 (also known as ADAMTS-4). Specifically, articular cartilage contains large amounts of the proteoglycan aggrecan. Proteoglycan aggrecan provides mechanical properties that help articular cartilage in withstanding compressive deformation during joint articulation. The loss of aggrecan fragments and their release into synovial fluid caused by proteolytic cleavages is a central pathophysiological event in osteoarthritis and rheumatoid arthritis. It has been reported that two major cleavage sites exist in the proteolytically sensitive

interglobular domains at the N-terminal region of the aggrecan core protein. One of those sites has been reported to be cleaved by several matrix metalloproteases. The other site, however, has been reported to be cleaved by aggrecanase-1. Thus, inhibiting excessive aggrecanase activity provides a method for preventing or

5   treating inflammatory conditions. *See generally*, Tang, B. L., "ADAMTS: A Novel Family of Extracellular Matrix Proteases," *Int'l Journal of Biochemistry & Cell Biology*, 33, pp. 33-44 (2001). Such diseases reportedly include, for example, osteoarthritis, rheumatoid arthritis, joint injury, reactive arthritis, acute pyrophosphate arthritis, and psoriatic arthritis. *See, e.g.*, European Patent

10   Application Publ. No. EP 1 081 137 A1.

[19]   In addition to inflammatory conditions, there also is evidence that inhibiting aggrecanase may be used for preventing or treating cancer. For example, excessive levels of aggrecanase-1 reportedly have been observed with a ghoma cell line. It also has been postulated that the enzymatic nature of aggrecanase and its

15   similarities with the MMPs would support tumor invasion, metastasis, and angiogenesis. *See* Tang, *Int'l Journal of Biochemistry & Cell Biology*, 33, pp. 33-44 (2001).

[20]   Various hydroxamate compounds have been reported to inhibit aggrecanase-1. Such compounds include, for example, those described in

20   European Patent Application Publ. No. EP 1 081 137 A1. Such compounds also include, for example, those described in WIPO PCT Int'l Publ. No. WO 00/09000. Such compounds further include, for example, those described in WIPO PCT Int'l Publ. No. WO 00/59874.

[21]   In view of the importance of hydroxamate compounds and salts

25   thereof in the prevention or treatment of several MMP- and/or aggrecanase-related pathological conditions and the lack of enzyme specificity exhibited by at least some of the hydroxamates that have been in clinical trials, there continues to be a need for hydroxamates having greater enzyme inhibition specificity (preferably toward MMP-2, MMP-9,MMP- 13, and/or aggrecanase, and particularly toward

MMP-13 and/or aggrecanase), while exhibiting little or no inhibition of MMP activity essential to normal bodily function (*e.g.*, tissue turnover and repair). The following disclosure describes hydroxamate compounds and salts thereof that tend to exhibit such desirable activities.

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SUMMARY OF THE INVENTION

[22] This invention is directed to compounds that inhibit MMP (particularly MMP-2, MMP-9, and/or MMP- 13) and/or aggrecanase activity, while generally exhibiting relatively little or no inhibition against MMP activity essential

10 to normal bodily function (particularly MMP-1 and MMP-14 activity). This invention also is directed to a method for inhibiting MMP and/or aggrecanase activity, particularly pathological activity. Such a method is particularly suitable to be used with mammals, such as humans, other primates (*e.g.*, monkeys, chimpanzees. etc.), companion animals (*e.g.*, dogs, cats, horses. etc.), farm animals

15 (*e.g.*, goats, sheep, pigs, cattle, etc.), laboratory animals (*e.g.*, mice, rats, etc.), and wild and zoo animals (*e.g.*, wolves, bears, deer, etc.).

[23] Briefly, therefore, the invention is directed in part to a compound or salt thereof. The compound has a structure corresponding to Formula X:
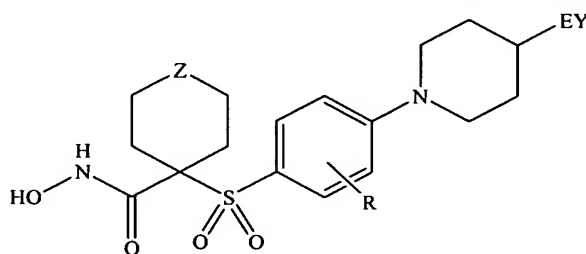
20   X.

The variables Z, R, E, and Y are described in more detail below.

[24] The present invention also is directed to treatment methods that comprise administering a compound described above (or pharmaceutically-acceptable salt thereof) in an effective amount to a host mammal having a

25 condition associated with pathological metalloprotease and/or aggrecanase activity.

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A contemplated compound or salt thereof tends to exhibit, for example, inhibitory activity of one or more matrix metalloprotease (MMP) enzymes (e.g., MMP-2, MMP-9 and MMP-13), while exhibiting substantially less inhibition of MMP-1 and/or MMP-14. By "substantially less" it is meant that a contemplated compound

5   exhibits an IC_{50} value ratio against one or more of MMP-2, MMP-9, or MMP-13 as compared to its IC_{50} value against MMP-1 and/or MMp-14 (e.g., IC_{50} MMP-13:IC_{50} MMP-1) that is less than about 1:10, preferably less than about 1:100, and most preferably less than about 1:1000 in the in vitro inhibition assay described in the Example section below.

10   **[25]**   In one embodiment, the process comprises administering an above-described compound or pharmaceutically acceptable salt thereof to the host animal in an amount effective to prevent or treat the condition. Such a condition may be, for example, tissue destruction, a fibrotic disease, pathological matrix weakening, defective injury repair, a cardiovascular disease, a pulmonary disease, a

15   kidney disease, and a central nervous system disease. Specific examples of such conditions include osteoarthritis, rheumatoid arthritis, septic arthritis, tumor invasion, tumor metastasis, tumor angiogenesis, a decubitis ulcer, a gastric ulcer, a corneal ulcer, periodontal disease, liver cirrhosis, fibrotic lung disease, otosclerosis, atherosclerosis, multiple sclerosis, dilated cardiomyopathy,

20   epidermolysis bullosa, aortic aneurysm, weak injury repair, an adhesion, scarring, congestive heart failure, coronary thrombosis, emphysema, proteinuria, and Alzheimer's disease.

[26]   In another embodiment, the prevention or treatment method comprises administering an above-described compound or pharmaceutically

25   acceptable salt thereof to the host animal in an amount effective to inhibit matrix metalloprotease-2, matrix metalloprotease-9, and/or matrix metalloprotease-13 activity.

[27]   In another embodiment, the prevention or treatment method comprises administering an above-described compound or pharmaceutically

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acceptable salt thereof to the host animal in an amount effective to prevent or treat a condition associated with TNF-α convertase activity. Examples of such a condition include inflammation, a pulmonary disease, a cardiovascular disease, an autoimmune disease, graft rejection, a fibrotic disease, cancer, an infectious

5   disease, fever, psoriasis, hemorrhage, coagulation, radiation damage, acute-phase responses of shock and sepsis, anorexia, and cachexia.

       [28]   In another embodiment, the prevention or treatment method comprises administering an above-described compound or pharmaceutically acceptable salt thereof to the host animal in an amount effective to prevent or treat

10   a condition associated with aggrecanase activity. Such a condition may be, for example, an inflammatory disease or cancer.

       [29]   This invention additionally is directed, in part, to pharmaceutical compositions comprising the above-described compounds or pharmaceutically acceptable salts thereof, and the use of those compositions in the above-described

15   prevention or treatment processes.

       [30]   This invention further is directed, in part, to the use of an above-described compound or pharmaceutically acceptable salt thereof for production of a medicament for use in the treatment of a condition related to MMP activity. As noted above, such a condition may be, for example, tissue destruction,

20   a fibrotic disease, pathological matrix weakening, defective injury repair, a cardiovascular disease, a pulmonary disease, a kidney disease, and a central nervous system disease.

       [31]   Further benefits of Applicants' invention will be apparent to one skilled in the art reading this patent.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

       [32]   This detailed description of preferred embodiments is intended only to acquaint others skilled in the art with Applicants' invention, its principles, and its practical application so that others skilled in the art may adapt and apply the

invention in its numerous forms, as they may be best suited to the requirements of a

particular use. This detailed description and its specific examples, while indicating

the preferred embodiments of this invention, are intended for purposes of

illustration only. This invention, therefore, is not limited to the preferred

5   embodiments described in this patent, and may be variously modified.

A. Compounds of This Invention

[33]   In accordance with this invention, Applicants have found that

certain aromatic sulfone hydroxamates tend to be effective toward inhibiting

10   MMPs, particularly those associated with excessive (or otherwise pathological)

breakdown of connective tissue. Specifically, Applicants have found that these

hydroxamates tend to be effective for inhibiting MMP-2 MMP-9, and/or MMP- 13,

which can be particularly destructive to tissue if present or generated in abnormally

excessive quantities or concentrations. Applicants also have discovered that many

15   of these hydroxamates tend to be effective toward inhibiting pathological

aggrecanase activity. Applicants have further discovered that these hydroxamates

tend to be selective toward inhibiting aggrecanase and/or MMPs associated with

pathological condition conditions, and tend to avoid excessive inhibition of MMPs

(particularly MMP-1 and MMP-14) essential to normal bodily function *(e.g.,* tissue

20   turnover and repair). Applicants have found, for example, that these hydroxamates

tend to be particularly active toward inhibiting MMP-2, MMP-9, MMP-13, and/or

aggrecanase activity in *in vitro* assays that are generally predictive of *in vivo*

activity, while exhibiting minimal inhibition toward MMP-1 and/or MMP-14 in

such assays. Examples of such *in vitro* assays are discussed in the example section

25   below. Compounds (or salts) that are particularly useful as selective MMP

inhibitors exhibit, for example, an *in vitro* IC_{50} value against one or more of

MMP-2, MMP-9, and MMP-13 that is no greater than about 0.1 times the IC_{50}

value against MMP-1 and/or MMP-14, more preferably no greater than about 0.01

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times the IC_{50} value against MMP-1 and/or MMP-14, and even more preferably

0.001 times the IC_{50} value against MMP-1 and/or MMP-14.

[34]   Without being bound by theory, the advantages of the selectivity of

a contemplated compound can be appreciated by considering the roles of the

5   various MMP and aggrecanase enzymes. For example, inhibition of MMP-1 is

believed to be undesirable due to the role of MMP-1 as a housekeeping enzyme

(*i.e.*, helping to maintain normal connective tissue turnover and repair). Inhibition

of MMP-1 can lead to toxicities or side effects such as such as joint or connective

tissue deterioration and pain. On the other hand, MMP-13 is believed to be

10   intimately involved in the destruction of joint components in diseases such as

osteoarthritis. Thus, potent and selective inhibition of MMP-13 is typically highly

desirable because such inhibition can have a positive effect on disease progression

in a patient (in addition to having an anti-inflammatory effect).

[35]   Another advantage of the compounds and salts of this invention is

15   their tendency to be selective with respect to tumor necrosis factor release and/or

tumor necrosis factor receptor release. This provides the physician with another

factor to help select the best drug for a particular patient. Without being bound by

theory, it is believed that there are multiple factors to this type of selectivity to be

considered. The first is that presence of tumor necrosis factor can be desirable for

20   the control of cancer in the organism, so long as TNF is not present in a toxic

excess. Thus, uncontrolled inhibition of release of TNF can be counterproductive

and actually can be considered an adverse side effect even in cancer patients. In

addition, selectivity with respect to inhibition of the release of the tumor necrosis

factor receptor can also be desirable. The presence of that receptor can be desirable

25   for maintaining a controlled tumor necrosis level in the mammal by binding excess

TNF.

[36]   Briefly, therefore, this invention is directed, in part, to a compound

or salt thereof (particularly a pharmaceutically acceptable salt thereof). The

compound has a structure corresponding to Formula X:
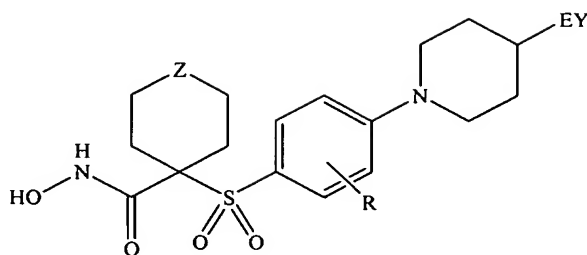
X.

[37]   In some preferred embodiments:

[38]   Z is -C(O)-, -N(R^6)-, -O-, -S-, -S(O)-, -S(O)$_2$-, or -N(S(O)$_2$$R^7$)-.

5   In some particularly preferred embodiments, Z is -O-. In other particularly

preferred embodiments, Z is -N(R^6)-.

[39] R^6 is hydrogen, formyl, sulfonic-C_1-C_6-alkyl, C_1-C_6-

alkoxycarbonyl-C_1-C_6-alkyl, carboxy-C_1-C_6-alkyl, C_1-C_6-alkylcarbonyl-C_1-C_6-

alkyl, R^8R^9-aminocarbonyl-C_1-C_6-alkyl, C_1-C_6-alkoxycarbonyl-C_1-C_6-

10   alkylcarbonyl, carboxy-C_1-C_6-alkylcarbonyl, C_1-C_6-alkylcarbonyl-C_1-C_6-

alkylcarbonyl, C_1-C_6-alkoxycarbonyl, carboxy, C_1-C_6-alkylcarbonyl, R^8R^9-

aminocarbonyl, aryl-C_1-C_6-alkyl, arylcarbonyl, bis(C_1-C_6-alkoxy-C_1-C_6-alkyl)-

C_1-C_6-alkyl, C_1-C_6-alkyl, halo-C_1-C_6-alkyl, trifluoromethyl-C_1-C_6-alkyl,

perfluoro-C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkyl, C_3-C_6-

15   cycloalkyl, heteroarylcarbonyl, heterocyclylcarbonyl, aryl, heterocyclyl, heteroaryl,

C_3-C_8-cycloalkyl-C_1-C_6-alkyl, aryloxy-C_1-C_6-alkyl, heteroaryloxy-C_1-C_6-alkyl,

heteroaryl-C_1-C_6-alkoxy-C_1-C_6-alkyl, heteroarylthio-C_1-C_6-alkyl, arylsulfonyl,

C_1-C_6-alkylsulfonyl, C_5-C_6-heteroarylsulfonyl, carboxy-C_1-C_6-alkyl,

aminocarbonyl, C_1-C_6-alkylimino(R^{10})carbonyl, arylimino(R^{10})carbonyl, C_5-C_6-

20   heterocyclylimino(R^{10})carbonyl, arylthio-C_1-C_6-alkyl, C_1-C_6-alkylthio-C_1-C_6-

alkyl, arylthio-C_3-C_6-alkenyl, C_1-C_4-alkylthio-C_3-C_6-alkenyl, C_5-C_6-heteroaryl-

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C_1-C_6-alkyl, halo-C_1-C_6-alkylcarbonyl, hydroxy-C_1-C_6-alkylcarbonyl, thiol-C_1-C_6-alkylcarbonyl, C_3-C_6-alkenyl, C_3-C_6-alkynyl, aryloxycarbonyl, R^8R^9-aminoimino(R^{10})methyl, R^8R^9-amino-C_1-C_5-alkylcarbonyl, hydroxy-C_1-C_5-alkyl, R^8R^9-aminocarbonyl, R^8R^9-aminocarbonyl-C_1-C_6-alkylcarbonyl,

5   hydroxyaminocarbonyl, R^8R^9-aminosulfonyl, R^8R^9-aminosulfonyl-C_1-C_6-alkyl, R^8R^9-amino-C_1-C_6-alkylsulfonyl, or R^8R^9-amino-C_1-C_6-alkyl.

[40]   In some particularly preferred embodiments, R^6 is C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkyl, C_3-C_6-cycloalkyl, C_3-C_8-cycloalkyl-C_1-C_6-alkyl, C_1-C_6-alkylsulfonyl, C_3-C_6-alkenyl, or C_3-C_6-alkynyl.

10   [41] R^7 is aryl-C_1-C_6-alkyl, aryl, heteroaryl, heterocyclyl, C_1-C_6-alkyl, C_3-C_6-alkynyl, C_3-C_6-alkenyl, carboxy-C_1-C_6-alkyl, or hydroxy-C_1-C_6-alkyl.

[42] R^8 and R^9 are independently selected from the group consisting of hydrogen, hydroxy, C_1-C_6-alkyl, C_1-C_6-alkylcarbonyl, arylcarbonyl, aryl, aryl-C_1-C_6-alkyl, heteroaryl, heteroaryl-C_1-C_6-alkyl, C_2-C_6-alkynyl, C_2-C_6-alkenyl, thiol-

15   C_1-C_6-alkyl, C_1-C_6-alkylthio-C_1-C_6-alkyl, cycloalkyl, cycloalkyl-C_1-C_6-alkyl, heterocyclyl-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkyl, aryl-C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkoxy-C_1-C_6-alkyl, hydroxy-C_1-C_6-alkyl, carboxy-C_1-C_6-alkyl, carboxyaryl-C_1-C_6-alkyl, aminocarbonyl-C_1-C_6-alkyl, aryloxy-C_1-C_6-alkyl, heteroaryloxy-C_1-C_6-alkyl, arylthio-C_1-C_6-alkyl, heteroarylthio-C_1-C_6-

20   alkyl, a sulfoxide of any said thio substituents, a sulfone of any said thio substituents, trifluoromethyl-C_1-C_6-alkyl, halo-C_1-C_6-alkyl, alkoxycarbonylamino-C_1-C_6-alkyl, and amino-C_1-C_6-alkyl. Here, the amino-C_1-C_6-alkyl nitrogen optionally is substituted with up to 2 substituents independently selected from the group consisting of C_1-C_6-alkyl, aryl-C_1-C_6-alkyl, cycloalkyl,

and C_1-C_6-alkylcarbonyl. Preferably, no greater than one of R^8 and R^9 is hydroxy.

[43] Alternatively, R^8 and R^9, together with the atom to which they are bonded, form a 5- to 8-membered heterocyclic or heteroaryl ring containing up to 2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

[44] R^{10} is hydrogen, hydroxy, C_1-C_6-alkyl, aryl, aryl-C_1-C_6-alkyl, heteroaryl, heteroaryl-C_1-C_6-alkyl, C_2-C_6-alkynyl, C_2-C_6-alkenyl, thiol-C_1-C_6-alkyl, C_1-C_6-alkylthio-C_1-C_6-alkyl, cycloalkyl, cycloalkyl-C_1-C_6-alkyl, heterocyclyl-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkyl, aryl-C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkoxy-C_1-C_6-alkyl, hydroxy-C_1-C_6-alkyl, carboxy-C_1-C_6-alkyl, carboxyaryl-C_1-C_6-alkyl, aminocarbonyl-C_1-C_6-alkyl, aryloxy-C_1-C_6-alkyl, heteroaryloxy-C_1-C_6-alkyl, arylthio-C_1-C_6-alkyl, heteroarylthio-C_1-C_6-alkyl, a sulfoxide of any said thio substituents, a sulfone of any said thio substituents, trifluoromethyl-C_1-C_6-alkyl, halo-C_1-C_6-alkyl, alkoxycarbonylamino-C_1-C_6-alkyl, and amino-C_1-C_6-alkyl. Here, the amino-C_1-C_6-alkyl nitrogen optionally is substituted with up to 2 substituents independently selected from the group consisting of C_1-C_6-alkyl, aryl-C_1-C_6-alkyl, cycloalkyl, and C_1-C_6-alkylcarbonyl.

[45] E is a bond, -C(O)-, or -S-.

[46] Y is hydrogen, alkyl, alkoxy, haloalkyl, aryl, arylalkyl, cycloalkyl, heteroaryl, hydroxy, aryloxy, arylalkoxy, heteroaryloxy, heteroarylalkyl, perfluoroalkoxy, perfluoroalkylthio, trifluoromethylalkyl, alkenyl, heterocyclyl, cycloalkyl, trifluoromethyl, alkoxycarbonyl, or aminoalkyl. Here, the aryl, heteroaryl, arylalkyl, or heterocyclyl optionally is substituted with up to 2 substituents independently selected from the group consisting of alkylcarbonyl,

15

halo, nitro, arylalkyl, aryl, alkoxy, trifluoroalkyl, trifluoroalkoxy, and amino. The

amino, in turn, optionally is substituted with up to 2 substituents independently

selected from the group consisting of alkyl and arylalkyl. In some particularly

preferred embodiments, Y comprises a cyclic structure, i.e., Y is optionally

5   substituted aryl, arylalkyl, cycloalkyl, heteroaryl, aryloxy, arylalkoxy,

heteroaryloxy, heteroarylalkyl, heterocyclyl, or cycloalkyl. In one such

embodiment, Y is optionally substituted phenyl. In another such embodiment, Y is

optionally substituted phenylmethyl. In still another such embodiment, Y is

optionally substituted heteraryl. And in still yet another such embodiment, Y is

10   optionally substituted heteroarylmethyl.

[47]   R is hydrogen, cyano, perfluoroalkyl, trifluoromethoxy,

trifluoromethylthio, haloalkyl, trifluoromethylalkyl, arylalkoxycarbonyl,

aryloxycarbonyl, hydroxy, halo, alkyl, alkoxy, nitro, thiol, hydroxycarbonyl,

aryloxy, arylthio, arylalkyl, aryl, arylcarbonylamino, heteroaryloxy, heteroarylthio,

15   heteroarylalkyl, cycloalkyl, heterocylyloxy, heterocylylthio, heterocylylamino,

cycloalkyloxy, cycloalkylthio, heteroarylalkoxy, heteroarylalkylthio, arylalkoxy,

arylalkylthio, arylalkylamino, heterocylyl, heteroaryl, arylazo,

hydroxycarbonylalkoxy, alkoxycarbonylalkoxy, alkylcarbonyl, arylcarbonyl,

arylalkylcarbonyl, alkylcarbonyloxy, arylalkylcarbonyloxy, hydroxyalkyl,

20   hydroxyalkoxy, alkylthio, alkoxyalkylthio, alkoxycarbonyl, aryloxyalkoxyaryl,

arylthioalkylthioaryl, aryloxyalkylthioaryl, arylthioalkoxyaryl,

hydroxycarbonylalkoxy, hydroxycarbonylalkylthio, alkoxycarbonylalkoxy,

alkoxycarbonylalkylthio, amino, aminocarbonyl, or aminoalkyl.

[48]   The nitrogen of an R amino may be unsubstituted. Alternatively,

25   the amino nitrogen may be substituted with up two substituents that are

independently selected from the group consisting of alkyl, aryl, heteroaryl,

arylalkyl, cycloalkyl, arylalkoxycarbonyl, alkoxycarbonyl, arylcarbonyl,

arylalkylcarbonyl, heteroarylcarbonyl, heteroarylalkylcarbonyl, and alkylcarbonyl.

Alternatively, the amino nitrogen optionally may be substituted with two

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substituents such that the two substituents, together with the amino nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl ring that: (i) contains from zero to two additional heteroatoms that are independently selected from the group consisting of nitrogen, oxygen, and sulfur; and (ii) optionally is substituted with up to two

5   substituents independently selected from the group consisting of aryl, alkyl, heteroaryl, arylalkyl, heteroarylalkyl, hydroxy, alkoxy, alkylcarbonyl, cycloalkyl, heterocylylalkyl, alkoxycarbonyl, hydroxyalkyl, trifluoromethyl, benzofused heterocylylalkyl, hydroxyalkoxyalkyl, arylalkoxycarbonyl, hydroxycarbonyl, aryloxycarbonyl, benzofused heterocylylalkoxy, benzofused cycloalkylcarbonyl,

10   heterocyclylalkylcarbonyl, and cycloalkylcarbonyl.

[49]   The nitrogen of an R aminocarbonyl is may be unsubstituted. Alternatively, the aminocarbonyl nitrogen may be the reacted amine of an amino acid. Alternatively, the aminocarbonyl nitrogen may be substituted with up to two substituents independently selected from the group consisting of alkyl,

15   hydroxyalkyl, hydroxyheteroarylalkyl, cycloalkyl, arylalkyl, trifluoromethylalkyl, heterocylylalkyl, benzofused heterocylylalkyl, benzofused cycloalkyl, and N,N-dialkylsubstituted alkylamino-alkyl. Alternatively, the aminocarbonyl nitrogen may be substituted with two substituents such that the two substituents, together with the aminocarbonyl nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl

20   ring that optionally is substituted with up to two substituents independently selected from the group consisting of alkyl, alkoxycarbonyl, nitro, heterocyclylalkyl, hydroxy, hydroxycarbonyl, aryl, arylalkyl, heteroaralkyl, and amino. Here, the amino nitrogen, in turn, optionally is substituted with: (i) two substituents independently selected from the group consisting of alkyl, aryl, and

25   heteroaryl; or (ii) two substituents such that the two substituents, together with the amino nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl ring.

[50]   The nitrogen of an R aminoalkyl may be unsubstituted. Alternatively, the aminoalkyl nitrogen may be substituted with up to two substituents independently selected from the group consisting of alkyl, aryl,

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arylalkyl, cycloalkyl, arylalkoxycarbonyl, alkoxycarbonyl, and alkylcarbonyl. Alternatively, the aminoalkyl nitrogen may be substituted with two substituents such that the two substituents, together with the aminoalkyl nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl ring.

5   [51]   In one particularly preferred embodiment, R is halogen (preferably chloro or fluoro, and even more preferably chloro). In another particularly preferred embodiment, R is hydrogen so that the compound corresponds in structure to Formula XA:

10   XA.
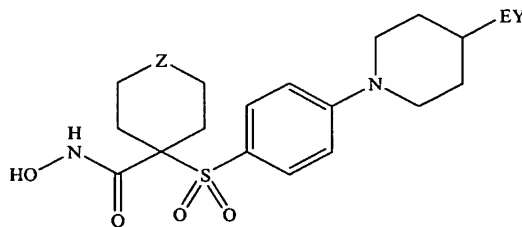
[52]   In other embodiments directed to compounds corresponding in structure to Formula X:

[53]   Z is -C(O)-, -N(R^6)-, -O-, -S-, or -S(O)$_2$-. In one particularly preferred embodiment, Z is -N(R^6)-. In another particularly preferred embodiment,
15   Z is -O-.

[54]   R^6 is hydrogen, arylalkoxycarbonyl, alkylcarbonyl, alkyl, alkoxyalkyl, cycloalkyl, heteroarylcarbonyl, heteroaryl, cycloalkylalkyl, alkylsulfonyl, haloalkylcarbonyl, alkenyl, alkynyl, and R^{8}R^9-aminoalkylcarbonyl.

[55]   In some particularly preferred embodiments, R^6 is hydrogen, aryl-
20   C$_1$-C$_6$-alkoxycarbonyl, C$_1$-C$_6$-alkoxycarbonyl, C$_1$-C$_6$-alkyl (preferably isopropyl), C$_1$-C$_6$-alkoxy-C$_1$-C$_6$-alkyl, C$_3$-C$_6$-cycloalkyl, heteroaryl, heteroarylcarbonyl, halo-C$_1$-C$_6$-alkylcarbonyl, or R^{8}R^9-amino-C$_1$-C$_6$-alkylcarbonyl.

[56]   In other particularly preferred embodiments, R^6 is C_1-C_6-alkyl

(preferably ethyl), C_1-C_6-alkoxy-C_1-C_6-alkyl (preferably methoxyethyl), C_3-C_6-

cycloalkyl (preferably cyclopropyl), C_3-C_8-cycloalkyl-C_1-C_6-alkyl (preferably

cyclopropylmethyl), C_3-C_6-alkenyl (preferably C_3-alkenyl), C_3-C_6-alkynyl

5   (preferably C_3-alkynyl), or C_1-C_6-alkylsulfonyl (preferably methylsulfonyl).

[57] R^8 and R^9 are independently selected from the group consisting of

hydrogen, alkyl, alkoxy, hydroxyalkyl, alkoxyalkyl, hydroxyalkoxyalkyl,

heteroarylalkyl, cycloalkylalkyl, heterocyclylcarbonyl, haloalkyl, and aminoalkyl.

Here, the aminoalkyl nitrogen optionally is substituted with up to two substituents

10   independently selected from the group consisting of alkyl.

[58]   Alternatively, R^8 and R^9, together with the atom to which they are

bonded, form a 5- to 8-membered heterocyclyl or heteroaryl containing up to 3 (in

many instances, no greater than 2) heteroatoms independently selected from the

group consisting of nitrogen, oxygen, and sulfur. Here, any such heterocyclyl or

15   heteroaryl (particularly heterocyclyl) optionally is substituted with one or more

substituents independently selected from the group consisting of hydroxy, keto,

carboxy, alkoxyalkyl, hydroxyalkyl, hydroxyalkoxyalkyl, alkoxycarbonylalkyl,

heterocyclylalkyl, alkoxycarbonyl, and aminoalkyl. The aminoalkyl nitrogen, in

turn, optionally is substituted with up to two substituents independently selected

20   from the group consisting of alkyl.

[59]   E is a bond, -C(O)-, or -S-.

[60]   Y is cycloalkyl, 2,3-dihydroindolyl, heterocyclyl, aryl, heteroaryl,

arylalkyl, or heteroarylalkyl. Here, the cycloalkyl, 2,3-dihydroindolyl, heterocyclyl,

aryl, heteroaryl, arylalkyl, or heteroarylalkyl optionally is substituted with one or

25   more substituents independently selected from the group consisting of halogen,

hydroxy, keto, alkyl, haloalkyl, hydroxyalkyl, alkenyl, alkoxy, alkylcarbonyl,

haloalkoxy, alkylthio, alkoxyalkyl, alkoxycarbonylalkyl, cycloalkyl,

cycloalkylalkyl, cycloalkyloxy, cycloalkylalkoxy, cycloalkylalkoxyalkyl, aryl,

arylalkyl, arylalkoxy, heterocyclyl, heterocyclylalkyl, heteroaryl,

heteroarylcarbonyl, heterocyclylcarbonylalkyl, alkylsulfonyl, amino, aminoalkyl,

and aminocarbonyl. These optional substituents, in turn, optionally are substituted

with one or more substituents independently selected from the group consisting of

5   halogen, nitro, alkyl, haloalkyl, alkoxy, haloalkoxy, and alkylcarbonyl.

Additionally, the nitrogen of the amino, aminoalkyl, or aminocarbonyl optionally is

substituted with up to two substituents independently selected from the group

consisting of alkyl and cycloalkylalkyl.

          [61]   In some preferred embodiments, E is -C(O)-, and Y is heterocyclyl,

10   aryl (particularly phenyl), heteroaryl, or arylmethyl (particularly phenylmethyl).

Here, the heterocyclyl, aryl, heteroaryl, or arylmethyl optionally is substituted with

one or more substituents independently selected from the group consisting of

halogen, hydroxy, C_1-C_6-alkyl, halo-C_1-C_6-alkyl, hydroxy-C_1-C_6-alkyl, C_2-C_6-

alkenyl, C_1-C_6-alkoxy, C_1-C_6-alkylcarbonyl, halo-C_1-C_6-alkoxy, C_1-C_6-alkylthio,

15   C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxycarbonyl-C_1-C_6-alkyl, C_3-C_6-cycloalkyl,

aryl, aryl-C_1-C_6-alkyl, aryl-C_1-C_6-alkoxy, heterocyclyl, heterocyclyl-C_1-C_6-alkyl,

heteroaryl, heteroarylcarbonyl, heterocyclylcarbonyl-C_1-C_6-alkyl, amino, and

amino-C_1-C_6-alkyl. These optional substituents, in turn, are optionally substituted

with one or more substituents independently selected from the group consisting of

20   halogen, nitro, C_1-C_6-alkyl, halo-C_1-C_6-alkyl, C_1-C_6-alkoxy, and C_1-C_6-

alkylcarbonyl. Additionally, the nitrogen of the amino or amino-C_1-C_6-alkyl

optionally is substituted with up to two substituents independently selected from

the group consisting of C_1-C_6-alkyl and C_3-C_6-cycloalkyl-C_1-C_6-alkyl.

          [62]   In other preferred embodiments, E is -C(O)-, and Y is aryl

25   (particularly phenyl), heteroaryl, arylmethyl (particularly phenylmethyl), or

heteroarylmethyl. The aryl, heteroaryl, arylmethyl, or heteroarylmethyl optionally

is substituted with one or more substituents independently selected from the group

consisting of halogen, C_1-C_6-alkyl, hydroxy-C_1-C_6-alkyl, C_2-C_6-alkenyl, C_1-C_6-

alkoxy, C_1-C_6-alkoxy-C_1-C_6-alkyl, C_3-C_6-cycloalkyl-C_1-C_6-alkyl, C_3-C_6-

cycloalkyloxy, C_3-C_6-cycloalkyl-C_1-C_6-alkoxy, C_3-C_6-cycloalkyl-C_1-C_6-alkoxy-C_1-C_6-alkyl, heterocyclyl-C_1-C_6-alkyl, amino, and amino-C_1-C_6-alkyl. And the nitrogen of the amino or amino-C_1-C_6-alkyl optionally is substituted with up to two substituents independently selected from the group consisting of C_1-C_6-alkyl. In

5   some such preferred embodiments, Y is optionally substituted phenyl. Such compounds include, for example:
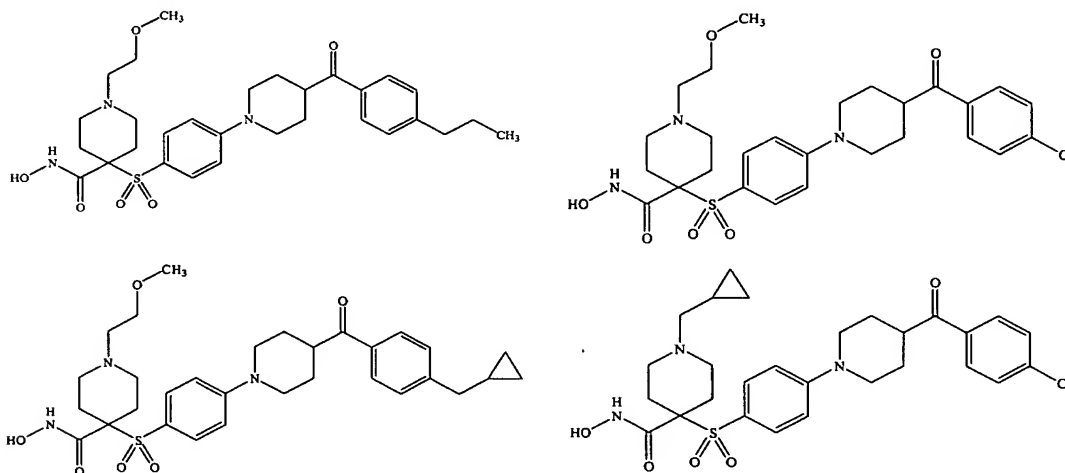
In other such preferred embodiments, Y is optionally substituted heteroaryl. Such compounds include, for example, compounds wherein Y is optionally substituted

10   thienyl:
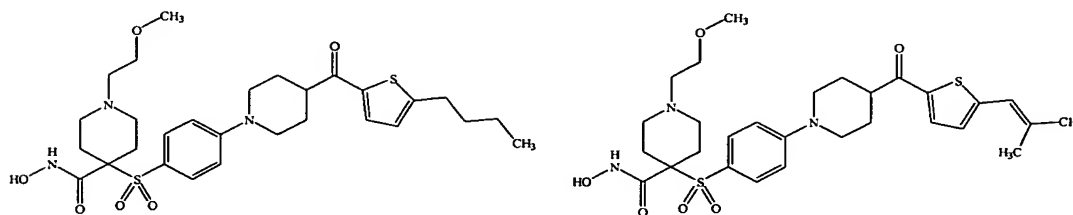
[63]   In other preferred embodiments, E is a bond, and Y is aryl (particularly phenyl), 2,3-dihydroindolyl, heterocyclyl, or heteroaryl. The aryl, 2,3-

15   dihydroindolyl, heterocyclyl, or heteroaryl optionally is substituted with one or more substituents independently selected from the group consisting of halogen,

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keto, hydroxy, C_1-C_6-alkyl, C_1-C_6-alkoxy, halo-C_1-C_6-alkyl, halo-C_1-C_6-alkoxy,

aryl, aminocarbonyl, and C_1-C_6-alkylsulfonyl. These optional substituents, in turn,

also are optionally substituted with one or more substituents independently selected

from the group consisting of halogen, halo-C_1-C_6-alkyl, and halo-C_1-C_6-alkoxy.

5   Additionally, the nitrogen of the aminocarbonyl optionally is substituted with up to

2 substituents independently selected from the group consisting of C_1-C_6-alkyl.

[64]   In other preferred embodiments, E is a bond, and Y is heteroaryl,

aryl (particularly phenyl), or heterocyclyl. The heteroaryl, aryl, or heterocyclyl

optionally is substituted with one or more substituents independently selected from

10   the group consisting of halogen, C_1-C_6-alkyl, C_1-C_6-alkoxy, and aryl. The

optional aryl substituent(s), in turn, optionally is/are substituted with one or more

substituents independently selected from the group consisting of halo-C_1-C_6-alkyl.

[65]   In other preferred embodiments, E is -S-, and Y is cycloalkyl, aryl,

arylmethyl, or heteroaryl. The cycloalkyl, aryl (particularly phenyl), arylmethyl

15   (particularly phenylmethyl), or heteroaryl optionally is substituted with one or more

substituents independently selected from the group consisting of halogen, halo-C_1-

C_6-alkyl, and halo-C_1-C_6-alkoxy.

[66]   In other preferred embodiments, E is -S-, and Y is heteraryl.

[67]   In the above embodiments, R preferably is halogen (preferably

20   chloro or fluoro, and even more preferably chloro). Alternatively, R preferably is

hydrogen so that the compound corresponds in structure to Formula XA (shown

above).

B. Preparation of Useful Compounds

25   [68]   Exemplary chemical transformations that can be useful for

preparing compounds and salts of this invention are described in detail in, for

example, WIPO Int'l Publ. Nos. WO 00/69821 (published November 23, 2000);

WO 00/50396 (published August 31, 2000); and 99/25687 (published May 27,

1999). These references are hereby incorporated by reference into this patent. The reader also is referred to the Example section below, which describes the preparation of numerous compounds and salts of this invention.

5 *C. Salts of the Compounds of this Invention*

[69] The compounds of this invention can be used in the form of salts derived from inorganic or organic acids. Depending on the particular compound, a salt of the compound may be advantageous due to one or more of the salt's physical properties, such as enhanced pharmaceutical stability in differing

10 temperatures and humidities, or a desirable solubility in water or oil. In some instances, a salt of a compound also may be used as an aid in the isolation, purification, and/or resolution of the compound.

[70] Where a salt is intended to be administered to a patient (as opposed to, for example, being used in an *in vitro* context), the salt preferably is

15 pharmaceutically acceptable. Pharmaceutically acceptable salts include salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. In general, these salts typically may be prepared by conventional means with a compound of this invention by reacting, for example, the appropriate acid or base with the compound.

20 [71] Pharmaceutically-acceptable acid addition salts of the compounds of this invention may be prepared from an inorganic or organic acid. Examples of suitable inorganic acids include hydrochloric, hydrobromic acid, hydroionic, nitric, carbonic, sulfuric, and phosphoric acid. Suitable organic acids generally include, for example, aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclyl,

25 carboxylic, and sulfonic classes of organic acids. Specific examples of suitable organic acids include acetate, trifluoroacetate, formate, propionate, succinate, glycolate, gluconate, digluconate, lactate, malate, tartaric acid, citrate, ascorbate, glucuronate, maleate, fumarate, pyruvate, aspartate, glutamate, benzoate, anthranilic acid, mesylate, stearate, salicylate, p-hydroxybenzoate, phenylacetate,

mandelate, embonate (pamoate), methanesulfonate, ethanesulfonate,

benzenesulfonate, pantothenate, toluenesulfonate, 2-hydroxyethanesulfonate,

sufanilate, cyclohexylaminosulfonate, algenic acid, b-hydroxybutyric acid,

galactarate, galacturonate, adipate, alginate, bisulfate, butyrate, camphorate,

5   camphorsulfonate, cyclopentanepropionate, dodecylsulfate, glycoheptanoate,

glycerophosphate, hemisulfate, heptanoate, hexanoate, nicotinate,

2-naphthalesulfonate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate,

picrate, pivalate, thiocyanate, tosylate, and undecanoate.

[72]   Pharmaceutically-acceptable base addition salts of the compounds

10   of this invention include, for example, metallic salts and organic salts. Preferred

metallic salts include alkali metal (group Ia) salts, alkaline earth metal (group IIa)

salts, and other physiological acceptable metal salts. Such salts may be made from

aluminum, calcium, lithium, magnesium, potassium, sodium, and zinc. Preferred

organic salts can be made from tertiary amines and quaternary amine salts, such as

15   tromethamine, diethylamine, N,N'-dibenzylethylenediamine, chloroprocaine,

choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), and

procaine. Basic nitrogen-containing groups can be quaternized with agents such as

lower alkyl (C_1-C_6) halides (e.g., methyl, ethyl, propyl, and butyl chlorides,

bromides, and iodides), dialkyl sulfates (e.g., dimethyl, diethyl, dibuytl, and diamyl

20   sulfates), long chain halides (e.g., decyl, lauryl, myristyl, and stearyl chlorides,

bromides, and iodides), aralkyl halides (e.g., benzyl and phenethyl bromides), and

others.

[73]   Particularly preferred salts of the compounds of this invention

include hydrochloric acid (HCl) salts and trifluoroacetate (CF_3COOH or TFA)

25   salts.

D. Preventing or Treating Conditions Using the Compounds and Salts of this Invention

[74]   One embodiment of this invention is directed to a process for preventing or treating a pathological condition associated with MMP activity in a

5   mammal (e.g., a human, companion animal, farm animal, laboratory animal, zoo animal, or wild animal) having or disposed to having such a condition. Such a condition may be, for example, tissue destruction, a fibrotic disease, pathological matrix weakening, defective injury repair, a cardiovascular disease, a pulmonary disease, a kidney disease, and a central nervous system disease. Specific examples

10   of such conditions include osteoarthritis, rheumatoid arthritis, septic arthritis, tumor invasion, tumor metastasis, tumor angiogenesis, a decubitis ulcer, a gastric ulcer, a corneal ulcer, periodontal disease, liver cirrhosis, fibrotic lung disease, otosclerosis, atherosclerosis, multiple sclerosis, dilated cardiomyopathy, epidermolysis bullosa, aortic aneurysm, weak injury repair, an adhesion, scarring,

15   congestive heart failure, coronary thrombosis, emphysema, proteinuria, and Alzheimer's disease.

[75]   The condition may alternatively (or additionally) be associated with TNF-α convertase activity. Examples of such a condition include inflammation (e.g., rheumatoid arthritis), autoimmune disease, graft rejection, multiple sclerosis,

20   a fibrotic disease, cancer, an infectious disease (e.g., malaria, mycobacterial infection, meningitis, etc.), fever, psoriasis, a cardiovascular disease (e.g., post-ischemic reperfusion injury and congestive heart failure), a pulmonary disease, hemorrhage, coagulation, hyperoxic alveolar injury, radiation damage, acute phase responses like those seen with infections and sepsis and during shock

25   (e.g., septic shock, hemodynamic shock, etc.), cachexia, and anorexia.

[76]   The condition may alternatively (or additionally) be associated with aggrecanase activity. Examples of such a condition include inflammation diseases (e.g., osteoarthritis, rheumatoid arthritis, joint injury, reactive arthritis, acute pyrophosphate arthritis, and psoriatic arthritis) and cancer.

[77]   In this patent, the phrase "preventing a condition" means reducing the risk of (or delaying) the onset of the condition in a mammal that does not have the condition, but is predisposed to having the condition. In contrast, the phrase "treating a condition" means ameliorating, suppressing, or eradicating an existing

5   condition. The pathological condition may be, for example: (a) the result of pathological MMP and/or aggrecanase activity itself, (b) affected by MMP activity *(e.g.,* diseases associated with TNF-α), and/or (c) affected by aggrecanase activity.

[78]   A wide variety of methods may be used alone or in combination to administer the hydroxamates and salt thereof described above. For example, the

10   hydroxamates or salts thereof may be administered orally, parenterally, by inhalation spray, rectally, or topically. Oral administration can be advantageous if, for example, the patient is ambulatory, not hospitalized, and physically able and sufficiently responsible to take drug at the required intervals. This may be true even if the person is being treated with more than one drug for one or more

15   diseases. On the other hand, IV drug administration can be advantageous in, for example, a hospital setting where the dose (and thus the blood levels) can be well controlled. A compound or salt of this invention also can be formulated for IM administration if desired. This route of administration may be desirable for administering prodrugs or regular drug delivery to patients that are either physically

20   weak or have a poor compliance record or require constant drug blood levels.

[79]   Typically, a compound (or pharmaceutically acceptable salt thereof) described in this patent is administered in an amount effective to inhibit a target MMP(s). The target MMP is/are typically MMP-2, MMP-9, and/or MMP-13, with MMP-13 often being a particularly preferred target. The preferred total daily dose

25   of the hydroxamate or salt thereof (administered in single or divided doses) is typically from about 0.001 to about 100 mg/kg, more preferably from about 0.001 to about 30 mg/kg, and even more preferably from about 0.01 to about 10 mg/kg *(i.e.,* mg hydroxamate or salt thereof per kg body weight). Dosage unit compositions can contain such amounts or submultiples thereof to make up the

daily dose. In many instances, the administration of the compound or salt will be repeated a plurality of times. Multiple doses per day typically may be used to increase the total daily dose, if desired.

[80]   Factors affecting the preferred dosage regimen include the type, age,

5   weight, sex, diet, and condition of the patient; the severity of the pathological condition; the route of administration; pharmacological considerations, such as the activity, efficacy, pharmacokinetic, and toxicology profiles of the particular hydroxamate or salt thereof employed; whether a drug delivery system is utilized; and whether the hydroxamate or salt thereof is administered as part of a drug

10   combination. Thus, the dosage regimen actually employed can vary widely, and, therefore, can deviate from the preferred dosage regimen set forth above.

E. Pharmaceutical Compositions Containing the Compounds and Salts of this Invention

15   [81]   This invention also is directed to pharmaceutical compositions comprising a hydroxamate or salt thereof described above, and to methods for making pharmacetucal compositions (or medicaments) comprising a hydroxamate or salt thereof described above.

[82]   The preferred composition depends on the method of

20   administration, and typically comprises one or more conventional pharmaceutically acceptable carriers, adjuvants, and/or vehicles. Formulation of drugs is generally discussed in, for example, Hoover, John E., *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, PA: 1975). See also, Liberman, H.A. *See also,* Lachman, L., eds., *Pharmaceutical Dosage Forms* (Marcel Decker, New York,

25   N.Y., 1980).

[83]   Solid dosage forms for oral administration include, for example, capsules, tablets, pills, powders, and granules. In such solid dosage forms, the hydroxamates or salts thereof are ordinarily combined with one or more adjuvants. If administered *per os*, the hydroxamates or salts thereof can be mixed with lactose,

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sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated

5   for convenient administration. Such capsules or tablets can contain a controlled-release formulation, as can be provided in a dispersion of the hydroxamate or salt thereof in hydroxypropylmethyl cellulose. In the case of capsules, tablets, and pills, the dosage forms also can comprise buffering agents, such as sodium citrate, or magnesium or calcium carbonate or bicarbonate. Tablets

10   and pills additionally can be prepared with enteric coatings.

[84]   Liquid dosage forms for oral administration include, for example, pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art (e.g., water). Such compositions also can comprise adjuvants, such as wetting, emulsifying,

15   suspending, flavoring (e.g., sweetening), and/or perfuming agents.

[85]   "Parenteral administration" includes subcutaneous injections, intravenous injections, intramuscular injections, intrasternal injections, and infusion. Injectable preparations (e.g., sterile injectable aqueous or oleaginous suspensions) can be formulated according to the known art using suitable

20   dispersing, wetting agents, and/or suspending agents. Acceptable vehicles and solvents include, for example, water, 1,3-butanediol, Ringer's solution, isotonic sodium chloride solution, bland fixed oils (e.g., synthetic mono- or diglycerides), fatty acids (e.g., oleic acid), dimethyl acetamide, surfactants (e.g., ionic and non-ionic detergents), and/or polyethylene glycols.

25   [86]   Formulations for parenteral administration may, for example, be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. The hydroxamates or salts thereof can be dissolved in water, polyethylene glycol,

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propylene glycol, ethanol, com oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers.

[87]   Suppositories for rectal administration can be prepared by, for example, mixing the drug with a suitable nonirritating excipient that is solid at ordinary temperatures, but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, such as cocoa butter; synthetic mono-, di-, or triglycerides; fatty acids; and/or polyethylene glycols

[88]   "Topical administration" includes the use of transdermal administration, such as transdermal patches or iontophoresis devices.

[89]   Other adjuvants and modes of administration known in the pharmaceutical art may also be used.

F. Definitions

[90]   The term "alkyl" (alone or in combination with another term(s)) means a straight-or branched-chain saturated hydrocarbyl group typically containing from 1 to about 20 carbon atoms, more typically from about 1 to about 8 carbon atoms, and even more typically from about 1 to about 6 carbon atoms. Examples of such groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl, and the like.

[91]   The term "alkenyl" (alone or in combination with another term(s)) means a straight- or branched-chain hydrocarbyl group containing one or more double bonds and typically from 2 to about 20 carbon atoms, more typically from about 2 to about 8 carbon atoms, and even more typically from about 2 to about 6 carbon atoms. Examples of such groups include ethenyl (vinyl); 2-propenyl; 3-propenyl; 1,4-pentadienyl; 1,4-butadienyl; 1-butenyl; 2-butenyl; 3-butenyl; decenyl; and the like.

[92]   The term "alkynyl" (alone or in combination with another term(s)) means a straight- or branched-chain hydrocarbyl group containing one or more

triple bonds and typically from 2 to about 20 carbon atoms, more typically from about 2 to about 8 carbon atoms, and even more typically from about 2 to about 6 carbon atoms. Examples of such groups include ethynyl, 2-propynyl, 3-propynyl, decynyl, 1-butynyl, 2-butynyl, 3-butynyl, and the like.

5   [93]   The term "carbocyclyl" (alone or in combination with another term(s)) means a saturated cyclic, partially saturated cyclic, or aryl hydrocarbyl group containing from 3 to 14 carbon ring atoms ("ring atoms" are the atoms bound together to form the ring or rings of a cyclic group). A carbocyclyl may be a single ring, which typically contains from 3 to 6 ring atoms. Examples of such

10   single-ring carbocyclyls include cyclopropanyl, cyclobutanyl, cyclopentyl, cyclopentenyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, and phenyl. A carbocyclyl alternatively may be 2 or 3 rings fused together, such as naphthalenyl, tetrahydronaphthalenyl (also known as "tetralinyl"), indenyl, isoindenyl, indanyl, bicyclodecanyl, anthracenyl, phenanthrene, benzonaphthenyl

15   (also known as "phenalenyl"), fluoreneyl, decalinyl, and norpinanyl.

        [94]   The term "cycloalkyl" (alone or in combination with another term(s)) means a saturated cyclic hydrocarbyl group containing from 3 to 14 carbon ring atoms. A cycloalkyl may be a single carbon ring, which typically contains from 3 to 6 carbon ring atoms. Examples of single-ring cycloalkyls include

20   cyclopropanyl, cyclobutanyl, cyclopentyl, and cyclohexyl. A cycloalkyl alternatively may be 2 or 3 carbon rings fused together, such as, decalinyl or norpinanyl.

        [95]   The term "aryl" (alone or in combination with another term(s)) means an aromatic carbocyclyl containing from 6 to 14 carbon ring atoms.

25   Examples of aryls include phenyl, naphthalenyl, and indenyl.

        [96]   In some instances, the number of carbon atoms in a hydrocarbyl group (e.g., alkyl, alkenyl, alkynyl, or cycloalkyl) is indicated by the prefix "C_x-C_y-", wherein x is the minimum and y is the maximum number of carbon atoms in the group. Thus, for example, "C_1-C_6-alkyl" refers to an alkyl group

containing from 1 to 6 carbon atoms. Illustrating further, C_3-C_6-cycloalkyl means a saturated hydrocarbyl ring containing from 3 to 6 carbon ring atoms.

[97] The term "hydrogen" (alone or in combination with another term(s)) means a hydrogen radical, and may be depicted as -H.

[98] The term "hydroxy" (alone or in combination with another term(s)) means -OH.

[99] The term "nitro" (alone or in combination with another term(s)) means -NO_2.

[100] The term "cyano" (alone or in combination with another term(s)) means -CN, which also may be depicted as or -COOH:

[101] The term "keto" (alone or in combination with another term(s)) means an oxo radical, and may be depicted as =O.

[102] The term "carboxy" (alone or in combination with another term(s)) means -C(O)-OH, which also may be depicted as:
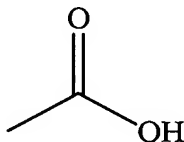
[103] The term "amino" (alone or in combination with another term(s)) means -NH_2. The term "monosubstituted amino" (alone or in combination with another term(s)) means an amino group wherein one of the hydrogen radicals is replaced by a non-hydrogen substituent. The term "disubstituted amino" (alone or in combination with another term(s)) means an amino group wherein both of the hydrogen atoms are replaced by non-hydrogen substituents, which may be identical or different.

[104] The term "halogen" (alone or in combination with another term(s)) means a fluorine radical (which may be depicted as -F), chlorine radical (which

31

may be depicted as -Cl), bromine radical (which may be depicted as -Br), or iodine radical (which may be depicted as -I). Typically, a fluorine radical or chlorine radical is preferred.

[105]   If a group is described as being "substituted", at least one hydrogen on the group is replaced with a non-hydrogen substituent. Thus, for example, a substituted alkyl group is an alkyl group wherein at least one hydrogen on the alkyl group is replaced with a non-hydrogen substituent. It should be recognized that if there are more than one substitutions on a group, each non-hydrogen substituent may be identical or different.

[106]   If a group is described as being "optionally substituted", the group may be either substituted or not substituted.

[107]   The prefix "halo" indicates that the group to which the prefix is attached is substituted with one or more independently selected halogen radicals. For example, haloalkyl means an alkyl group wherein at least one hydrogen radical is replaced with a halogen radical. Examples of haloalkyls include chloromethyl, 1-bromoethyl, fluoromethyl, difluoromethyl, trifluoromethyl, 1,1,1-trifluoroethyl, and the like. Illustrating further, "haloalkoxy" means an alkoxy group wherein at least one hydrogen radical is replaced by a halogen radical. Examples of haloalkoxy groups include chlormethoxy, 1-bromoethoxy, fluoromethoxy, difluoromethoxy, trifluoromethoxy (also known as "perfluoromethyoxy"), 1,1,1,-trifluoroethoxy, and the like. It should be recognized that if a group is substituted by more than one halogen radical, those halogen radicals may be identical or different.

[108]   The prefix "perhalo" indicates that every hydrogen radical on the group to which the prefix is attached is replaced with independently selected halogen radicals, i.e., each hydrogen radical on the group is replaced with a halogen radical. If all the halogen radicals are identical, the prefix typically will identify the halogen radical. Thus, for example, the term "perfluoro" means that every hydrogen radical on the group to which the prefix is attached is substituted

with a fluorine radical. To illustrate, the term "prefluoroalkyl" means an alkyl group wherein each hydrogen radical is replaced with a fluorine radical. Examples of perfluoroalkyl groups include trifluoromethyl ($-CF_3$), perfluorobutyl, perfluoroisopropyl, perfluorododecyl, perfluorodecyl, and the like. To illustrate

5   further, the term "perfluoroalkoxy" means an alkoxy group wherein each hydrogen radical is replaced with a fluorine radical. Examples of perfluoroalkoxy groups include trifluoromethoxy ($-O-CF_3$), perfluorobutoxy, perfluoroisopropoxy, perfluorododecoxy, perfluorodecoxy, and the like.

[109]   The term "carbonyl" (alone or in combination with another term(s))

10   means $-C(O)-$, which also may be depicted as:
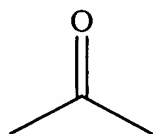
This term also is intended to encompass a hydrated carbonyl group, i.e., $-C(OH)_2-$.

[110]   The term "aminocarbonyl" (alone or in combination with another term(s)) means $-C(O)-NH_2$, which also may be depicted as:

15
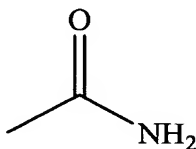
[111]   The term "oxy" (alone or in combination with another term(s)) means an ether group, and may be depicted as $-O-$.

[112]   The term "alkoxy" (alone or in combination with another term(s)) means an alkylether group, i.e., $-O$-alkyl. Examples of such a group include

20   methoxy ($-O-CH_3$), ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy, and the like.

[113]   The term "alkylcarbonyl" (alone or in combination with another term(s)) means $-C(O)$-alkyl. For example, "ethylcarbonyl" may be depicted as:
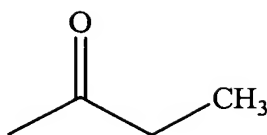
[114] The term "aminoalkylcarbonyl" (alone or in combination with another term(s)) means -C(O)-alkyl-NH_2. For example, "aminomethylcarbonyl" may be depicted as:
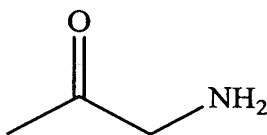
5

[115] The term "alkoxycarbonyl" (alone or in combination with another term(s)) means -C(O)-O-alkyl. For example, "ethoxycarbonyl" may be depicted as:
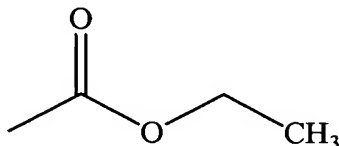
[116] The term "carbocyclylcarbonyl" (alone or in combination with another term(s)) means -C(O)-carbocyclyl. For example, "phenylcarbonyl" may be
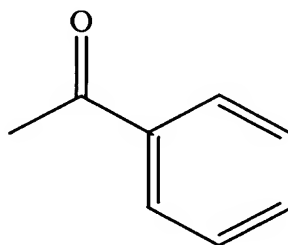
10 depicted as:

Similarly, the term "heterocyclylcarbonyl" (alone or in combination with another term(s)) means -C(O)-heterocyclyl.

15 **[117]** The term "carbocyclylalkylcarbonyl" (alone or in combination with another term(s)) means -C(O)-alkyl-carbocyclyl. For example, "phenylethylcarbonyl" may be depicted as:
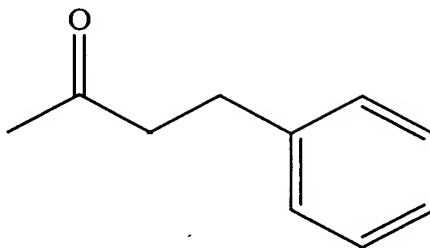
Similarly, the term "heterocyclylalkylcarbonyl" (alone or in combination with another term(s)) means -C(O)-alkyl-heterocyclyl.

[118] The term "carbocyclyloxycarbonyl" (alone or in combination with another term(s)) means -C(O)-O-carbocyclyl. For example, "phenyloxycarbonyl" may be depicted as:
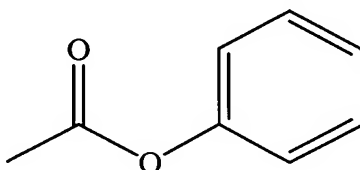
[119] The term "carbocyclylalkoxycarbonyl" (alone or in combination with another term(s)) means -C(O)-O-alkyl-carbocyclyl. For example, "phenylethoxycarbonyl" may be depicted as:
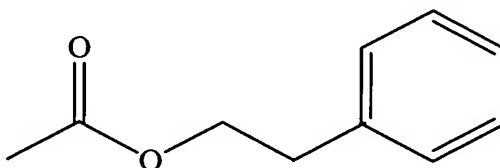
[120] The term "thio" or "thia" (alone or in combination with another term(s)) means a thiaether group, i.e., an ether group wherein the ether oxygen atom is replaced by a sulfur atom. Such a group may be depicted as -S-. This, for example, "alkyl-thio-alkyl" means alkyl-S-alkyl.

[121] The term "thiol" or "sulfhydryl" (alone or in combination with another term(s)) means a sulfhydryl group, and may be depicted as -SH.

[122] The term "(thiocarbonyl)" (alone or in combination with another term(s)) means a carbonyl wherein the oxygen atom has been replaced with a sulfur. Such a group may be depicted as -C(S)-, and also may be depicted as:

35
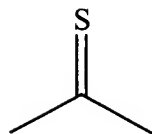
[123] The term "alkyl(thiocarbonyl)" (alone or in combination with another term(s)) means -C(S)-alkyl. For example, "ethyl(thiocarbonyl)" may be depicted as:
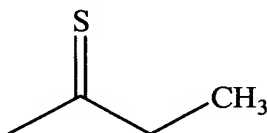
5

[124] The term "alkoxy(thiocarbonyl)" (alone or in combination with another term(s)) means -C(S)-O-alkyl. For example, "ethoxy(thiocarbonyl)" may be depicted as:
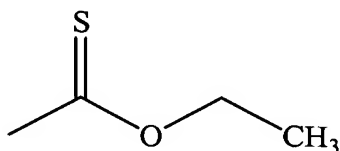
10   **[125]** The term "carbocyclyl(thiocarbonyl)" (alone or in combination with another term(s)) means -C(S)-carbocyclyl. For example, "phenyl(thiocarbonyl)" may be depicted as:
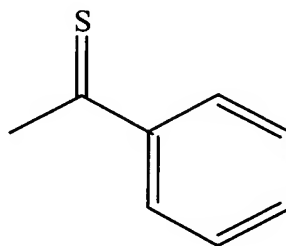
Similarly, the term "heterocyclyl(thiocarbonyl)" (alone or in combination with
15   another term(s)) means -C(S)-heterocyclyl.

     [126] The term "carbocyclylalkyl(thiocarbonyl)" (alone or in combination with another term(s)) means -C(S)-alkyl-carbocyclyl. For example, "phenylethyl(thiocarbonyl)" may be depicted as:
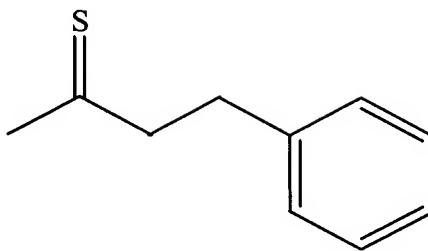
Similarly, the term "heterocyclylalkyl(thiocarbonyl)" (alone or in combination with another term(s)) means -C(S)-alkyl-heterocyclyl.

[127]   The term "carbocyclyloxy(thiocarbonyl)" (alone or in combination

5   with another term(s)) means -C(S)-O-carbocyclyl. For example, "phenyloxy(thiocarbonyl)" may be depicted as:
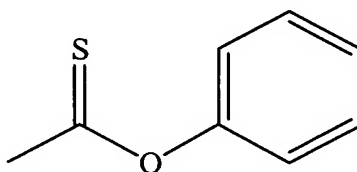
[128]   The term "carbocyclylalkoxy(thiocarbonyl)" (alone or in combination with another term(s)) means -C(S)-O-alkyl-carbocyclyl. For example,

10   "phenylethoxy(thiocarbonyl)" may be depicted as:
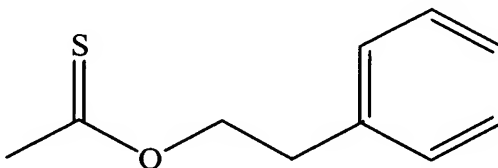
[129]   The term "sulfonyl" (alone or in combination with another term(s)) means -S(O)$_2$-, which also may be depicted as:
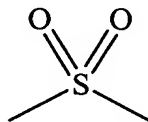
15   Thus, for example, "alkyl-sulfonyl-alkyl" means alkyl-S(O)$_2$-alkyl.

[130]   The term "aminosulfonyl" (alone or in combination with another term(s)) means -S(O)$_2$-NH$_2$, which also may be depicted as:

37
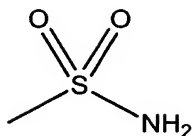
[131] The term "sulfoxido" (alone or in combination with another term(s)) means -S(O)-, which also may be depicted as:
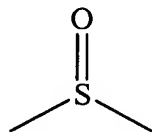
5   Thus, for example, "alkyl-sulfoxido-alkyl" means alkyl-S(O)-alkyl.

[132] The term "heterocyclyl" (alone or in combination with another term(s)) means a saturated or partially saturated ring structure containing a total of 3 to 14 ring atoms. At least one of the ring atoms is a heteroatom (i.e., oxygen, nitrogen, or sulfur), with the remaining ring atoms being independently selected

10 from the group consisting of carbon, oxygen, nitrogen, and sulfur. A heterocyclyl may be a single ring, which typically contains from 3 to 7 ring atoms, more typically from 3 to 6 ring atoms, and even more typically 5 to 6 ring atoms. A heterocyclyl alternatively may be 2 or 3 rings fused together.

[133] The term "heteroaryl" (alone or in combination with another

15 term(s)) means an aromatic ring containing from 5 to 14 ring atoms. At least one of the ring atoms is a heteroatom, with the remaining ring atoms being independently selected from the group consisting of carbon, oxygen, nitrogen, and sulfur. A heteroaryl may be a single ring, which typically contains from 5 to 7 ring atoms, and more typically from 5 to 6 ring atoms. A heteroaryl alternatively may

20 be 2 or 3 rings fused together.

[134] Examples of single-ring heterocyclyls and heteroaryls include furanyl, dihydrofurnayl, tetradydrofurnayl, thiophenyl (also known as "thiofuranyl"), dihydrothiophenyl, tetrahydrothiophenyl, pyrrolyl, isopyrrolyl, pyrrolinyl, pyrrolidinyl, imidazolyl, isoimidazolyl, imidazolinyl, imidazolidinyl,

25 pyrazolyl, pyrazolinyl, pyrazolidinyl, triazolyl, tetrazolyl, dithiolyl, oxathiolyl,

oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, thiazolinyl, isothiazolinyl, thiazolidinyl, isothiazolidinyl, thiodiazolyl, oxathiazolyl, oxadiazolyl (including 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl (also known as "azoximyl"), 1,2,5-oxadiazolyl (also known as "furazanyl"), or 1,3,4-oxadiazolyl), oxatriazolyl (including

5   1,2,3,4-oxatriazolyl or 1,2,3,5-oxatriazolyl), dioxazolyl (including 1,2,3-dioxazolyl, 1,2,4-dioxazolyl, 1,3,2-dioxazolyl, or 1,3,4-dioxazolyl), oxathiazolyl, oxathiolyl, oxathiolanyl, pyranyl (including 1,2-pyranyl or 1,4-pyranyl), dihydropyranyl, pyridinyl (also known as "azinyl"), piperidinyl, diazinyl (including pyridazinyl (also known as "1,2-diazinyl"), pyrimidinyl (also

10 known as "1,3-diazinyl"), or pyrazinyl (also known as "1,4-diazinyl")), piperazinyl, triazinyl (including s-triazinyl (also known as "1,3,5-triazinyl"), as-triazinyl (also known 1,2,4-triazinyl), and v-triazinyl (also known as "1,2,3-triazinyl")), oxazinyl (including 1,2,3-oxazinyl, 1,3,2-oxazinyl, 1,3,6-oxazinyl (also known as "pentoxazolyl"), 1,2,6-oxazinyl, or 1,4-oxazinyl), isoxazinyl (including

15 o-isoxazinyl or p-isoxazinyl), oxazolidinyl, isoxazolidinyl, oxathiazinyl (including 1,2,5-oxathiazinyl or 1,2,6-oxathiazinyl), oxadiazinyl (including 1,4,2-oxadiazinyl or 1,3,5,2-oxadiazinyl), morpholinyl, azepinyl, oxepinyl, thiepinyl, and diazepinyl.

[135]   Examples of heterocyclyl and heteroaryl rings having 2 or 3 rings fused together include, for example, indolizinyl, pyrindinyl, pyranopyrrolyl,

20 4H-quinolizinyl, purinyl, naphthyridinyl, pyridopyridinyl (including pyrido[3,4-b]-pyridinyl, pyrido[3,2-b]-pyridinyl, or pyrido[4,3-b]-pyridinyl), and pteridinyl. Other examples of fused-ring heterocyclyls include benzo-fused heterocyclyls, such as indolyl, isoindolyl (also known as "isobenzazolyl" or "pseudoisoindolyl"), indoleninyl (also known as "pseudoindolyl"), isoindazolyl

25 (also known as "benzpyrazolyl"), benzazinyl (including quinolinyl (also known as "1-benzazinyl") or isoquinolinyl (also known as "2-benzazinyl")), phthalazinyl, quinoxalinyl, quinazolinyl, benzodiazinyl (including cinnolinyl (also known as "1,2-benzodiazinyl") or quinazolinyl (also known as "1,3-benzodiazinyl")), benzopyranyl (including "chromanyl" or "isochromanyl"), benzothiopyranyl (also

known as "thiochromanyl"), benzoxazolyl, indoxazinyl (also known as
"benzisoxazolyl"), anthranilyl, benzodioxolyl, benzodioxanyl, benzoxadiazolyl,
benzofuranyl (also known as "coumaronyl"), isobenzofuranyl, benzothienyl (also
known as "benzothiophenyl", "thionaphthenyl", or "benzothiofuranyl"),

5   isobenzothienyl (also known as "isobenzothiophenyl", "isothionaphthenyl", or
"isobenzothiofuranyl"), benzothiazolyl, benzothiadiazolyl, benzimidazolyl,
benzotriazolyl, benzoxazinyl (including 1,3,2-benzoxazinyl , 1,4,2-benzoxazinyl ,
2,3,1-benzoxazinyl , or 3,1,4-benzoxazinyl), benzisoxazinyl (including
1,2-benzisoxazinyl or 1,4-benzisoxazinyl), tetrahydroisoquinolinyl , carbazolyl,

10   xanthenyl, and acridinyl.

       [136] As may be seen in the preceding paragraphs, the term "heteroaryl"
includes 6-membered ring substituents such as pyridyl, pyrazyl, pyrimidinyl, and
pyridazinyl; 5-membered ring substituents such as 1,3,5-, 1,2,4- or 1,2,3-tiiazinyl,
imidazyl, furanyl, thiophenyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, 1,2,3-,

15   1,2,4-, 1,2,5-, or 1,3,4-oxadiazolyl and isothiazolyl; 6/5-membered fused ring
substituents such as benzothiofuranyl, isobenzothiofuranyl, benzisoxazolyl,
benzoxazolyl, purinyl, and anthranilyl; and 6/6-membered fused rings such as 1,2-,
1,4-, 2,3- and 2, 1-benzopyronyl, quinolinyl, isoquinolinyl, cinnolinyl,
quinazolinyl, and 1,4-benzoxazinyl.

20   **[137]** A carbocyclyl, heterocyclyl, or heteroaryl optionally can be
substituted with, for example, one or more substituents independently selected
from the group consisting of halogen, hydroxy, carboxy, keto, alkyl, alkoxy,
alkoxyalkyl, alkylcarbonyl (also known as "alkanoyl"), aryl, arylalkyl, arylalkoxy,
arylalkoxyalkyl, arylalkoxycarbonyl, cycloalkyl, cycloalkylalkyl, cycloalkylalkoxy,

25   cycloalkylalkoxyalkyl, and cycloalkylalkoxycarbonyl. More typically, a
carbocyclyl or heterocyclyl may optionally be substituted with, for example, one or
more substituents independently selected from the group consisting of halogen,
-OH, -C(O)-OH, keto, C_1-C_6-alkyl, C_1-C_6-alkoxy, C_1-C_6-alkoxy-C_1-C_6-alkyl,
C_1-C_6-alkylcarbonyl, aryl, aryl-C_1-C_6-alkyl, aryl-C_1-C_6-alkoxy,

aryl-C_1-C_6-alkoxy-C_1-C_6-alkyl, aryl-C_1-C_6-alkoxycarbonyl, cycloalkyl,

cycloalkyl-C_1-C_6-alkyl, cycloalkyl-C_1-C_6-alkoxy,

cycloalkyl-C_1-C_6-alkoxy-C_1-C_6-alkyl, and cycloalkyl-C_1-C_6-alkoxycarbonyl. The

alkyl, alkoxy, alkoxyalkyl, alkylcarbonyl, aryl, arylalkyl, arylalkoxy,

5   arylalkoxyalkyl, or arylalkoxycarbonyl substituent(s) may further be substituted

with, for example, one or more halogen. The aryls or cycloalkyls are typically

single-ring groups containing from 3 to 6 ring atoms, and more typically from 5 to

6 ring atoms.

[138]   An aryl or heteroaryl optionally can be substituted with, for

10   example, one or more substituents independently selected from the group

consisting of halogen, -OH, -CN, -NO_2, -SH, -C(O)-OH, amino, aminocarbonyl,

aminoalkyl, alkyl, alkylthio, carboxyalkylthio, alkylcarbonyl, alkylcarbonyloxy,

alkoxy, alkoxyalkyl, alkoxycarbonyl, alkoxycarbonylalkoxy, alkoxyalkylthio,

alkoxycarbonylalkylthio, carboxyalkoxy, alkoxycarbonylalkoxy, carbocyclyl,

15   carbocyclylalkyl, carbocyclyloxy, carbocyclylthio, carbocyclylalkylthio,

carbocyclylamino, carbocyclylalkylamino, carbocyclylcarbonylamino,

carbocyclylcarbonyl, carbocyclylalkyl, carbonyl, carbocyclylcarbonyloxy,

carbocyclyloxycarbonyl, carbocyclylalkoxycarbonyl,

carbocyclyloxyalkoxycarbocyclyl, carbocyclylthioalkylthiocarbocyclyl,

20   carbocyclylthioalkoxycarbocyclyl, carbocyclyloxyalkylthiocarbocyclyl,

heterocyclyl, heterocyclylalkyl, heterocyclyloxy, heterocyclylthio,

heterocyclylalkylthio, heterocyclylamino, heterocyclylalkylamino,

heterocyclylcarbonylamino, heterocyclylcarbonyl, heterocyclylalkylcarbonyl,

heterocyclyloxycarbonyl, heterocyclylcarbonyloxy, heterocyclylalkoxycarbonyl,

25   heterocyclyloxyalkoxyheterocyclyl, heterocyclylthioalkylthioheterocyclyl,

heterocyclylthioalkoxyheterocyclyl, and heterocyclyloxyalkylthioheterocyclyl.

More typically, an aryl or heteroaryl may, for example, optionally be substituted

with one or more substituents independently selected from the group consisting of

halogen, -OH, -CN, -NO_2, -SH, -C(O)-OH, amino, aminocarbonyl,

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amino-C_1-C_6-alkyl, C_1-C_6-alkyl, C_1-C_6-alkylthio, carboxy-C_1-C_6-alkylthio,

C_1-C_6-alkylcarbonyl, C_1-C_6-alkylcarbonyloxy, C_1-C_6-alkoxy,

C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxycarbonyl,

C_1-C_6-alkoxycarbonyl-C_1-C_6-alkoxy, C_1-C_6-alkoxy-C_1-C_6-alkylthio,

5   C_1-C_6-alkoxycarbonyl-C_1-C_6-alkylthio, carboxy-C_1-C_6-alkoxy,

C_1-C_6-alkoxycarbonyl-C_1-C_6-alkoxy, aryl, aryl-C_1-C_6-alkyl, aryloxy, arylthio,

aryl-C_1-C_6-alkylthio, arylamino, aryl-C_1-C_6-alkylamino, arylcarbonylamino,

arylcarbonyl, aryl-C_1-C_6-alkylcarbonyl, arylcarbonyloxy, aryloxycarbonyl,

aryl-C_1-C_6-alkoxycarbonyl, aryloxy-C_1-C_6-alkoxyaryl, arylthio-C_1-C_6-alkylthioaryl,

10   arylthio-C_1-C_6-alkoxyaryl, aryloxy-C_1-C_6-alkylthioaryl, cycloalkyl,

cycloalkyl-C_1-C_6-alkyl, cycloalkyloxy, cycloalkylthio, cycloalkyl-C_1-C_6-alkylthio,

cycloalkylamino, cycloalkyl-C_1-C_6-alkylamino, cycloalkylcarbonylamino,

cycloalkylcarbonyl, cycloalkyl-C_1-C_6-alkylcarbonyl, cycloalkylcarbonyloxy,

cycloalkyloxycarbonyl, cycloalkyl-C_1-C_6-alkoxycarbonyl, heteroaryl,

15   heteroaryl-C_1-C_6-alkyl, heteroaryloxy, heteroarylthio, heteroaryl-C_1-C_6-alkylthio,

heteroarylamino, heteroaryl-C_1-C_6-alkylamino, heteroarylcarbonylamino,

heteroarylcarbonyl, heteroaryl-C_1-C_6-alkylcarbonyl, heteroaryloxycarbonyl,

heteroarylcarbonyloxy, and heteroaryl-C_1-C_6-alkoxycarbonyl. Here, one or more

hydrogens bound to a carbon in any such group may, for example, optionally be

20   replaced with halogen. In addition, the cycloalkyl, aryl, and heteroaryl are typically

single-ring groups containing 3 to 6 ring atoms, and more typically 5 or 6 ring

atoms.

[139]   In some embodiments, an aryl or heteroaryl optionally is substituted

with one or more substituents independently selected from the group consisting of

25   cyano, perfluoroalkyl, trifluoromethoxy, trifluoromethylthio, haloalkyl,

trifluoromethylalkyl, aralkoxycarbonyl, aryloxycarbonyl, hydroxy, halo, alkyl,

alkoxy, nitro, thiol, hydroxycarbonyl, aryloxy, arylthio, aralkyl, aryl,

arylcarbonylamino, heteroaryloxy, heteroarylthio, heteroaralkyl, cycloalkyl,

heterocylyloxy, heterocylylthio, heterocylylamino, cycloalkyloxy, cycloalkylthio,

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heteroaralkoxy, heteroaralkylthio, aralkoxy, aralkylthio, aralkylamino, heterocylyl, heteroaryl, arylazo, hydroxycarbonylalkoxy, alkoxycarbonylalkoxy, alkanoyl, arylcarbonyl, aralkanoyl, alkanoyloxy, aralkanoyloxy, hydroxyalkyl, hydroxyalkoxy, alkylthio, alkoxyalkylthio, alkoxycarbonyl, aryloxyalkoxyaryl,

5   arylthioalkylthioaryl, aryloxyalkylthioaryl, arylthioalkoxyaryl, hydroxycarbonylalkoxy, hydroxycarbonylalkylthio, alkoxycarbonylalkoxy, alkoxycarbonylalkylthio, amino, aminocarbonyl, and aminoalkyl. Here, the amino nitrogen optionally is substituted with:

         (i)   up two substituents that are independently selected from the group

10   consisting of alkyl, aryl, heteroaryl, aralkyl, cycloalkyl, aralkoxycarbonyl, alkoxycarbonyl, arylcarbonyl, aralkanoyl, heteroarylcarbonyl, heteroaralkanoyl, and alkanoyl; or

         (ii)   two substituents such that the two substituents, together with the amino nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl

15   ring that:

                 (a)   contains from zero to two additional heteroatoms that are independently selected from the group consisting of nitrogen, oxygen, and sulfur;

                 (b)   optionally is substituted with up to two substituents

20   independently selected from the group consisting of aryl, alkyl, heteroaryl, aralkyl, heteroaralkyl, hydroxy, alkoxy, alkanoyl, cycloalkyl, heterocylylalkyl, alkoxycarbonyl, hydroxyalkyl, trifluoromethyl, benzofused heterocylylalkyl, hydroxyalkoxyalkyl, aralkoxycarbonyl, hydroxycarbonyl,

25   aryloxycarbonyl, benzofused heterocylylalkoxy, benzofused cycloalkylcarbonyl, heterocyclylalkylcarbonyl, and cycloalkylcarbonyl.

The aminocarbonyl nitrogen is:

         (i)   unsubstituted;

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(ii)   the reacted amine of an amino acid;

(iii)   substituted with one or two substituents independently selected from the group consisting of alkyl, hydroxyalkyl, hydroxyheteroaralkyl, cycloalkyl, aralkyl, trifluoromethylalkyl, heterocylylalkyl, benzofused heterocylylalkyl, benzofused cycloalkyl, and N,N-dialkylsubstituted alkylaminoalkyl; or

(iv)   substituted with two substituents such that the two substituents, together with the aminocarbonyl nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl ring that optionally is substituted with up to two substituents independently selected from the group consisting of alkyl, alkoxycarbonyl, nitro, heterocylylalkyl, hydroxy, hydroxycarbonyl, aryl, aralkyl, heteroaralkyl, and amino, wherein the amino nitrogen optionally is substituted with:

    (a)   two substituents independently selected from the group consisting of alkyl, aryl, and heteroaryl; or

    (b)   two substituents such that the two substituents, together with the amino nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl ring.

The aminoalkyl nitrogen optionally is substituted with:

(i)   up to two substituents independently selected from the group consisting of alkyl, aryl, aralkyl, cycloalkyl, aralkoxycarbonyl, alkoxycarbonyl, and alkanoyl; or

(ii)   two substituents such that the two substituents, together with the aminoalkyl nitrogen, form a 5- to 8-member heterocyclyl or heteroaryl ring.

[140]   A prefix attached to a multi-component group only applies to the first component. To illustrate, the term "alkylcycloalkyl" contains two components: alkyl and cycloalkyl. Thus, the C_1-C_6- prefix on C_1-C_6-alkylcycloalkyl means that the alkyl component of the alkylcycloalkyl

contains from 1 to 6 carbon atoms; the C_1-C_6- prefix does not describe the cycloalkyl component. To illustrate further, the prefix "halo" on haloalkoxyalkyl indicates that *only* the alkoxy component of the alkoxyalkyl group is substituted with one or more halogen radicals. If halogen substitution may *alternatively or additionally* occur on the alkyl component, the group would instead be described as "halogen-substituted alkoxyalkyl" rather than "haloalkoxyalkyl." And finally, if the halogen substitution may *only* occur on the alkyl component, the group would instead be described as "alkoxyhaloalkyl."

[141] If substituents are described as being "independently selected" from a group, each substituent is selected independent of the other. Each substituent therefore may be identical to or different from the other substituent(s).

[142] When words are used to describe a substituent, the rightmost-described component of the substituent is the component that is bound at the location of the replaced hydrogen. To illustrate, benzene substituted with methoxyethyl has the following structure:
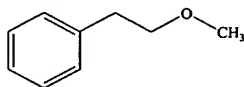
As can be seen, the ethyl is bound to the benzene, and the methoxy is the component of the substituent that is the component furthest from the benzene. As further illustration, benzene substituted with cyclohexanylthiobutoxy has the following structure:
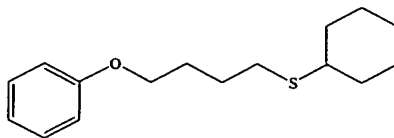
[143] When words are used to describe a linking element between two other elements of a depicted chemical structure, the rightmost-described component of the substituent is the component that is bound to the left element in the depicted structure. To illustrate, if the chemical structure is X-L-Y and L is

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described as methylcyclohexanylethyl, the chemical would be
X-ethyl-cyclohexanyl-methyl-Y.

[144]   When a chemical formula is used to describe a substituent, the dash
on the left side of the formula indicates the portion of the substituent that is bound

5   at the location of the replaced hydrogen. To illustrate, benzene substituted with
-C(O)-OH has the following structure:
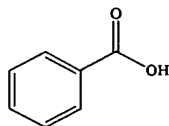
[145]   When a chemical formula is used to describe a linking element
between two other elements of a depicted chemical structure, the leftmost dash of

10   the substituent indicates the portion of the substituent that is bound to the left
element in the depicted structure. The rightmost dash, on the other hand, indicates
the portion of the substituent that is bound to the right element in the depicted
structure. To illustrate, if the depicted chemical structure is X-L-Y and L is
described as -C(O)-N(H)-, the chemical would be:
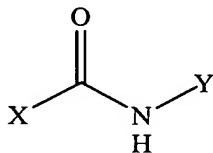
15

[146]   The term "pharmaceutically acceptable" is used adjectivally in this
patent to mean that the modified noun is appropriate for use as a pharmaceutical
product or as a part of a pharmaceutical product.

[147]   With reference to the use of the words "comprise" or "comprises" or

20   "comprising" in this patent (including the claims), Applicants note that unless the
context requires otherwise, those words are used on the basis and clear
understanding that they are to be interpreted inclusively, rather than exclusively,
and that Applicants intend each of those words to be so interpreted in construing
this patent, including the claims below.

EXAMPLES

[148] The following examples are merely illustrative, and not intended to be limiting to the remainder of this disclosure in any way.

[149] Abbreviations are often used for reagents and solvents in the specific examples that follow. Those abbreviations include the following:

BOC = *t*-butoxycarbonyl

DEAD = diethyl azodicarboxylate

DMF = dimethylformamide

DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone

EtOAc = ethyl acetate

EDC = 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride

Et_2O = diethyl ether

HOBT = 1-hydroxybenzotriazole

MeOH = methanol

$MeCl_2$ = methylene chloride

MsCl = methanesulfonyl chloride

NMM = N-methyl morpholine

THF = tetrahydrofruan

TsCl = toluenesulfonyl chloride

THP-O-hydroxylamine = O-tetrahydropyran-hydroxylamine and O-tetrahydro-2H-pyran-2-yl-hydroxylamine

[150] The preparation of compounds useful in the synthesis of compounds of the invention are provided herein below in Preparative Examples I through XI.

[151] Preparative Example I: Preparation of 1,1-dimethylethyl ester 4-[(hydroxyamino)-carbonyl]-4-[(phenoxyphenyl)-sulfonyl]-1-piperidinecarboxylic acid
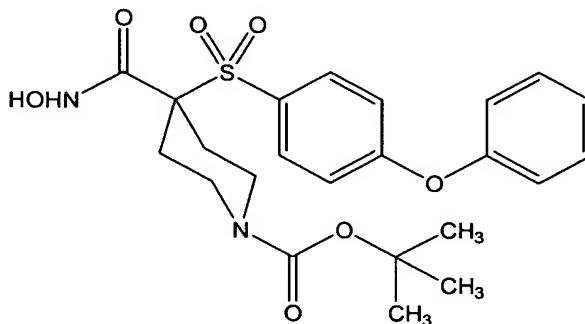
[152] Part A: A solution of 4-(phenoxy)benzenethiol (2.03 g, 10.0 mmol) in DMSO (DMSO; 20 mL) was heated to 65°C for 5 hr. The solution remained at ambient temperature for 18 hr. The solution was extracted with ethyl acetate and the combined organic layers were washed with H_2O and saturated NaCl and dried over magnesium sulfate. Concentration *in vacuo* provided the disulfide as a yellow oil (2.3 g, quantitative yield).

[153] Part B: To a solution of ethyl isonipecotate (15.7 g, 0.1 mol) in THF (100 mL) was added a solution of di-tert-butyl dicarbonate (21.8 g, 0.1 mol) in THF (5 mL) drop-wise over 20 min. The solution was stirred overnight (about 18 °C) at ambient temperature and concentrated *in vacuo* to yield a light oil. The oil was filtered through silica gel (7:3 ethyl acetate/hexanes) and concentrated *in vacuo* to give the BOC-piperidine compound (26.2 g, quantitative yield) as a clear, colorless oil.

[154] Part C: To a solution of diisopropylamine (2.8 mL, 20 mmoL) in THF (30 mL), cooled to -78°C, was added n-butyl lithium (12.5 mL, 20 mmol) drop-wise. After 15 min, the BOC-piperidine compound of part B (2.6 g, 10 mmol) in THF (10 mL) was added drop-wise. After 1.5 hr, the solution was cooled to -60°C and the disulfide of part A (2.0 g, 10 mmol) in THF (7 mL). The solution was stirred at ambient temperature for 2 hr. The solution was diluted with H_2O and extracted with ethyl acetate. The organic layer was washed with H_2O and saturated

NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the sulfide as an oil (1.8 g, 40%).

[155]   Part D: To a solution of the sulfide of part C (1.8 g, 3.95 mmol) in dichloromethane (75 mL) cooled to 0°C, was added m-chloroperbenzoic acid (1.7 g, 7.9 mmol). The solution was stirred for 1.5 hr followed by dilution with H_2O and extraction with dichloromethane. The organic layer was washed with 10 percent Na_2SO_4, H_2O, and saturated NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the sulfone as a solid (1.15 g, 59%).

[156]   Part E: To a solution of the sulfone of part D (800 mg, 1.63 mmol) in THF (9 mL) and ethanol (9 mL) was added NaOH (654 mg, 16.3 mmol) in H_2O (3 mL). The solution was heated at 65°C for 18 hr. The solution was concentrated *in vacuo* and the residue was dissolved in H_2O. Following acidification with 2N HCl to pH 4, the solution was extracted with ethyl acetate and the organic layer was washed with saturated NaCl and dried over magnesium sulfate. Concentration *in vacuo* provided the acid as a white foam (790 mg, quantitative yield). Analytical calculated for $C_{23}H_{27}NO_7S$: C, 59.86; H, 5.90; N, 3.04; S, 6.95. Found: C, 59.49; H, 6.37; N, 2.81; S, 6.59.

[157]   Part F: To a solution of the acid of part G (730 mg, 1.58 mmol) in DMF (9 mL) was added HOBT (256 mg, 1.90 mmol) followed by EDC (424 mg, 2.21 mmol), 4-methylmorpholine (0.521 mL, 4.7 mmol) and 50 percent aqueous hydroxylamine (1.04 mL, 15.8 mmol). The solution was stirred for 20 hr and additional N-hydroxybenzotriazole•H_2O (256 mg), EDC (424 mg) and 50 percent aqueous hydroxylamine (1.04 mL) were added. After an additional 24 hr of stirring, the solution was diluted with H_2O and extracted with ethyl acetate and the organic layer was washed with saturated NaCl and dried over magnesium sulfate. Reverse phase chromatography (on silica, acetonitrile/H_2O) provided the title compound as a white solid (460 mg, 61%). HPLC purity: >99%. Analytical

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calculated for $C_{23}H_{28}N_2O_7S$: C, 57.97; H, 5.92; N, 5.88; S, 6.73. Found: C, 57.95; H, 6.02; N, 5.81; S, 6.85.

[158] Preparative Example II: Preparation of N-hydroxy-4-[[4-
(phenylthio)phenyl]sulfonyl]-1-(2-propynyl)-4-piperidinecarboxamide,
monohydrochloride
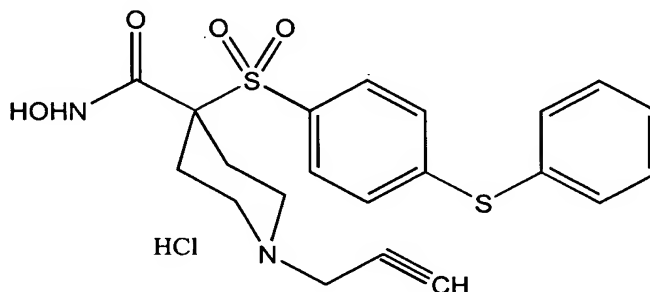
[159] Part A: To a solution of ethyl isonipecotate (15.7 g, 0.1 mol) in THF (100 mL) was added a solution of di-tert-butyl dicarbonate (21.8 g, 0.1 mol) in THF (5 mL) drop-wise over 20 min. The solution was stirred overnight (about 18 hr) at ambient temperature and concentrated *in vacuo* to yield a light oil. The oil was filtered through silica gel (ethyl acetate/hexanes) and concentrated *in vacuo* to give the BOC-piperidine compound as a clear, colorless oil (26.2 g, quantitative yield).

[160] Part B: A solution of 4-fluorothiophenol (50.29 g, 390 mmol) in DMSO (500 mL) was heated to 65°C for 6 hr. The reaction was quenched into wet ice and the resulting solid was collected by vacuum filtration to provide the disulfide as a white solid (34.4 g, 68.9%).

[161] Part C: To a solution of the BOC-piperdine compound of part A (16 g, 62 mmol) in THF (300 mL) cooled to minus 50°C was added lithium diisopropylamide (41.33 mL, 74 mmol) and the solution was stirred for 1.5 hr at 0°C. To this solution was added the disulfide of part B (15.77 g, 62 mmol), and the resulting solution was stirred at ambient temperature for 20 hr. The reaction was quenched with the addition of H_2O and the solution was concentrated *in vacuo*.

The aqueous residue was extracted with ethyl acetate and the organic layer was washed with 0.5N KOH, H_2O, and saturated NaCl. Chromatography (on silica, hexane/ethyl acetate) provided the sulfide as an oil (18.0 g, 75%).

[162] Part D: To a solution of the sulfide of part C (16.5 g, 43 mmol) in dichloromethane (500 mL) cooled to 0°C was added 3-chloroperbenzoic acid (18.0 g, 86 mmol) and the solution was stirred for 20 hr. The solution was diluted with H_2O and extracted with dichloromethane. The organic layer was washed with 10 percent Na_2SO_3, H_2O, and saturated NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the sulfone as a solid (10.7 g, 60%).

[163] Part E: Into a solution of the sulfone of part D (10 g, 24.0 mmol) in ethyl acetate (250 mL) was bubbled HCl gas for 10 min followed by stirring at ambient temperature for 4 hr. Concentration *in vacuo* provided the amine hydrochloride salt as a white solid (7.27 g, 86%).

[164] Part F: To a solution of the amine hydrochloride salt of part E (5.98 g, 17.0 mmol) in DMF (120 mL) was added potassium carbonate (4.7 g, 34.0 mmol) followed by propargyl bromide (2.02 g, 17.0 mmol) and the solution was stirred for 4 hr at ambient temperature. The solution was partitioned between ethyl acetate and H_2O, and the organic layer was washed with H_2O and saturated NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the propargyl amine as a yellow oil (5.2 g, 86%).

[165] Part G: To a solution of the propargyl amine of part F in DMF (15 mL) was added thiophenol (0.80 mL, 7.78 mmol) and $CsCO_3$ (2.79 g, 8.56 mmol) and the solution was heated to 70°C for 6 hr. The solution was partitioned between ethyl ether and H_2O. The organic layer was washed with H_2O and saturated NaCl, and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the S-phenoxyphenyl compound as an oil (1.95 g, 56%).

[166] Part H: To a solution of the S-phenoxyphenyl of part G (1.81 g, 4.06 mmol) in ethanol (21 mL) and H_2O (3.5 mL) was added KOH (1.37 g, 24.5

mmol) and the solution was heated to 105°C for 4.5 hr. The solution was acidified to a pH value of 1 with concentrated HCl solution and then concentrated to provide the acid as a yellow residue that was used without additional purification (1.82 g).

[167] Part I: To a solution of the acid of part H (1.82 g, 4.06 mmol) in acetonitrile (20 mL) was added O-tetrahydro-2H-pyran-2-yl-hydroxylamine (723 mg, 6.17 mmol) and triethylamine (0.67 mL, 4.86 mmol). To this stirring solution was added EDC (1.18 g, 6.17 mmol) and the solution was stirred for 18 hr. The solution was partitioned between H₂O and ethyl acetate. The organic layer was washed with H₂O, saturated NaHCO₃ and saturated NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the protected hydroxamate as a white solid (1.32 g, 63%).

[168] Part J: To a solution of the protected hydroxamate of part I (9.65 g, 18.7 mmol) in methanol (148 mL) cooled to 0°C was added acetyl chloride (4.0 mL, 56.2 mmol), and the solution was stirred for 45 min at ambient temperature. Concentration *in vacuo* followed by trituration with ethyl ether provided the title compound as a white solid (8.10 g, 94%). MS(CI) MH⁺ calculated for $C_{21}H_{22}N_2O_4S_2$: 431, found 431.

[169] **Preparative Example III: Preparation of N-hydroxy-4-[(4-phenoxyphenyl)sulfonyl]-1-(2-propynyl)-4-piperidinecarboxamide, monohydrochloride**
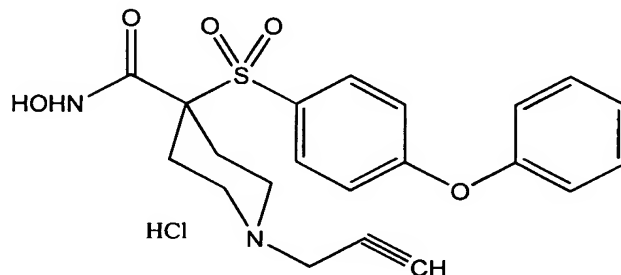
[170] Part A: A solution of 4-(phenoxy)benzenethiol (2.03 g, 10.0 mmol) in DMSO (20 mL) was heated to 65°C for 5 hr. The solution remained at ambient temperature for 18 hr. The solution was extracted with ethyl acetate and the

combined organic layers were washed with H_2O and saturated NaCl, and dried over magnesium sulfate. Concentration *in vacuo* provided the disulfide as a yellow oil (2.3 g, quantitative yield).

[171]   Part B: To a solution of ethyl isonipecotate (15.7 g, 0.1 mol) in THF (100 mL) was added a solution of di-tert-butyl dicarbonate (21.8 g, 0.1 mol) in THF (5 mL) dropwise over 20 min. The solution was stirred overnight at ambient temperature and concentrated *in vacuo* to yield a light oil. The oil was filtered through silica gel (ethyl acetate/hexane) and concentrated *in vacuo* to give the BOC-piperidine compound as a clear, colorless oil (26.2 g, quantitative yield).

[172]   Part C: To a solution of diisopropylamine (2.8 mL, 20 mmoL) in THF (30 mL), cooled to -78°C, was added n-butyl lithium (12.5 mL, 20 mmol) dropwise. After 15 min, the BOC-piperidine compound of Part B (2.6 g, 10 mmol) in THF (10 mL) was added dropwise. After 1.5 hr, the solution was cooled to -60°C and the disulfide of Part A (2.0 g, 10 mmol) in THF (7 mL) was added. The solution was stirred at ambient temperature for 2 hr. The solution was diluted with H_2O and extracted with ethyl acetate. The organic layer was washed with H_2O and saturated NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the sulfide as an oil (1.8 g, 40%).

[173]   Part D: To a solution of the sulfide of Part C (1.8 g, 3.95 mmol) in dichloromethane (75 mL) cooled to 0°C, was added m-chloroperbenzoic acid (1.7 g, 7.9 mmol). The solution was stirred for 1.5 hr followed by dilution with H_2O and extraction with dichloromethane. The organic layer was washed with 10 percent Na_2SO_4, H_2O, and saturated NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the sulfone as a solid (1.15 g, 59%).

[174]   Part E: Into a solution of the sulfone of Part D (3.56 g, 7.0 mmol) in ethyl acetate (100 mL) cooled to 0°C was bubbled HCl gas for 5 min. Concentration *in vacuo* followed by trituration with ethyl ether provided the amine

hydrochloride salt as a white solid (3.5 g, quantitative yield). MS(CI) MH^+ calculated for $C_{20}H_{23}NO_5S$: 390, found 390.

[175] Part F: To a solution of the amine hydrochloride salt of part E (2.6 g, 6 mmol) and K_2CO_3 (1.66 g, 12 mmol) in DMF (50 mL) was added propargyl bromide (892 mg, 6 mmol) and the solution was stirred at ambient temperature for 4 hr. The solution was diluted with H_2O and extracted with ethyl acetate. The combined organic layers were washed with saturated NaCl and dried over magnesium sulfate. Chromatography (on silica, ethyl acetate/hexane) provided the propargyl amine as a white solid (2.15 g, 82%).

[176] Part G: To a solution of the propargyl amine of part F (2.15 g, 5 mmol) in THF (30 mL) and ethanol (30 mL) was added NaOH (2.0 g, 50 mmol) and the solution was heated at 65°C for 48 hr. The solution was concentrated *in vacuo* and the aqueous residue was acidified to a pH value of 5. Vacuum filtration of the resulting precipitate provided the acid as a white solid (2.04 g, quantitative yield).

[177] Part H: To a solution of the acid of part G (559 mg, 1.4 mmol) in dichloromethane (5 mL) was added triethylamine (0.585 mL, 4.2 mmol) and 50 percent aqueous hydroxylamine (0.925 mL, 14.0 mmol) followed by bromotris(pyrrolidino)phosphonium hexafluourphosphate (PyBroP®; 718 mg, 1.54 mmol). The solution was stirred at ambient temperature for 4 hr. The solution was diluted with H_2O and extracted with dichloromethane. The organic layer was washed with saturated NaCl and dried over magnesium sulfate. Reverse phase chromatography (on silica, acetonitrile/H_2O) provided the hydroxamate as a white solid (140 mg, 25%). Analytical calculation for $C_{21}H_{22}N_2O_5S$: C, 60.85; H, 5.37; N, 6.76; S, 7.74. Found: C, 60.47; H, 5.35; N, 6.61; S, 7.46.

[178] Part I: To a solution of the hydroxamate of part H (121 mg, 0.292 mmol) in methanol (2 mL) cooled to 0°C was added acetyl chloride (0.228 mL, 0.321 mmol) in methanol (1 mL). After stirring at ambient temperature for 30 min, the solution was concentrated under a stream of N_2. Trituration with ethyl ether

provided the title compound as a white solid (107 mg, 81%). Analytical

calculation for $C_{21}H_{22}N_2O_5S \cdot HCl \cdot 0.3H_2O$: C, 55.27; H, 5.21; N, 6.14. Found: C,

54.90; H, 5.37; N, 6.07.

5   **[179]   Preparative Example IV: Preparation of 4-[(4-**
fluorophenyl)sulfonyl]tetrahydro-N-[(tetrahydro-2H-pyran-2-yl)oxy]-2H-
pyran-4-carboxamide
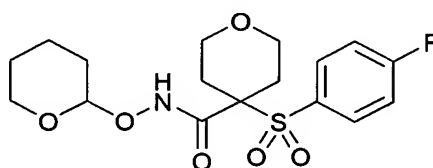
[180]   Part A: In dry equipment under nitrogen, sodium metal (8.97 g,

10   0.39 mol) was added to methanol (1000 mL) at 2°C. The reaction was stirred at

ambient temperature for 45 min, at which time the sodium had dissolved. The

solution was chilled to 5°C and p-fluorothiophenol (41.55 mL, 0.39 mmol) was

added, followed by methyl 2-chloroacetate (34.2 mL, 0.39 mol). The reaction was

stirred at ambient temperature for 4 hr, filtered, and concentrated *in vacuo* to give

15   the sulfide as a clear colorless oil (75.85 g, 97%).

[181]   Part B: To a solution of the sulfide from part A (75.85 g, 0.38 mol)

in methanol (1000 mL) were added water (100 mL) and Oxone®(720 g, 1.17 mol)

at 20°C. An exotherm to 67°C was noted. After 2 hr, the reaction was filtered and

the cake was washed well with methanol. The filtrate was concentrated *in vacuo*.

20   The residue was taken up in ethyl acetate and washed with brine, dried over

$MgSO_4$, filtered, and concentrated *in vacuo* to give the sulfone as a crystalline solid

(82.74 g, 94%).

[182]   Part C: To a solution of the sulfone from part B (28.5 g, 0.123 mol)

in N,N-dimethylacetamide (200 mL) were added potassium carbonate (37.3 g, 0.27

25   mol), bis-(2-bromoethyl)ether (19.3 mL, 0.147 mol), 4-dimethylaminopyridine

(0.75 g, 6 mmol), and tetrabutylammonium bromide (1.98 g, 6 mmol). The

reaction was stirred overnight (about 18 hr) at ambient temperature. The reaction

was slowly poured into 1N HCl (300 mL), the resultant solid filtered and the cake
washed well with hexanes. The solid was recrystallized from ethyl acetate/hexanes
to give the pyran compound as a beige solid (28.74 g, 77%). MS (ES+) MH+
calculated for $C_{13}H_{15}O_5S_1F_1$: 303, found 303.

[183] Part D: In dry equipment under nitrogen, the pyran compound from
part C (8.0 g, 26.5 mmol) was dissolved in dry tetrahydrofuran (250 mL) and a
solution of potassium trimethylsilonate (10.2 g, 79.5 mmol) in dry tetrahydrofuran
(15 mL) was added at ambient temperature. After 90 min, water (100 mL) was
added and the solution concentrated *in vacuo*. The residue was taken up in water
and extracted with ethyl acetate to remove unreacted starting material. The
aqueous solution was treated with 6N HCl until pH=1. The slurry was extracted
with ethyl acetate and the combined extracts washed with water, dried over
Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was heated in diethyl
ether, the solid filtered and dried to give the carboxylic acid as a crystalline solid
(5.78 g, 76%). HRMS (ES-) M-H calculated for $C_{12}H_{13}O_5$ S_1F_1: 287.04, found
287.04.

[184] Part E: In dry equipment under nitrogen, the carboxylic acid from
part D (9.1g, 31.6 mmol) was dissolved in dry N,N-dimethylformamide (70 mL)
and the remaining reagents were added to the solution in the following order: N-
hydroxybenzotriazole hydrate (5.1 g, 37.9 mmol), N-methylmorpholine (10.4 mL,
94.8 mmol), O-tetrahydro-2H-pyran-2-yl-hydroxylamine (11.5 g, 98 mmol), and 1-
(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (8.48 g, 44.2 mmol).
After 3 hr at ambient temperature, the reaction was concentrated *in vacuo*. The
residue was taken up in ethyl acetate, washed with water, 5% KHSO₄, saturated
NaHCO₃, brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*.
Chromatography (on silica, ethyl acetate/hexanes) provided the title compound as a
crystalline solid (9.7 g, 80%). HRMS (ES+) MH+ calculated for $C_{17}H_{22}NO_6$ S_1F_1:
388.12, found 388.12.

[185]   Preparative Example V: Preparation of tetrahydro-N-hydroxy-4-[[4-[4-(4-trifluoromethoxy)-phenoxy)phenyl]sulfonyl]-2H-pyran-4-carboxamide
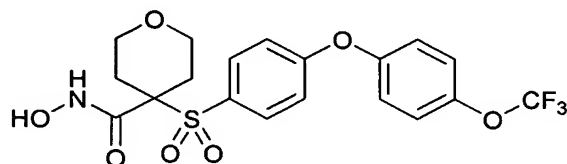
5   **[186]**   Part A: To a solution of the title compound of Preparative Example IV (3.1 g, 8 mmol) in N,N-dimethylacetamide (20 mL) were added cesium carbonate (8.8 g, 27 mmol) and p-(trifluoromethoxy)phenol (2.1 mL, 16 mmol). The slurry was stirred at 95°C for 19 hr. The reaction was concentrated *in vacuo*. The residue was taken up in ethyl acetate, washed with brine, dried over Na_2SO_4,

10   filtered, and concentrated *in vacuo*. Chromatography (on silica, ethyl acetate/hexanes) provided the substituted THP-protected hydroxamate as a white foam (4.2 g, 96%). HRMS (ES+) MH+ calculated for $C_{24}H_{26}N_1O_8 S_1F_3$:   546.14, found 546.14.

          [187]   Part B: To a slurry of the THP-protected hydroxamate from part A

15   (4.0 g, 7.3 mmol) in dioxane (20 mL) were added a 4N HCl dioxane solution (20 mL) and methanol (20 mL). After 15 min, at ambient temperature, the reaction was diluted with ethyl acetate and washed with water, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The product was recrystallized (acetone/hexanes) to give the title compound as a white solid (2.2 g, 65%). HRMS (ES+) M+ NH$_4$ $^+$

20   calculated for $C_{19}H_{18}N_1O_7S_1F_3$: 479.11, found 479.11.

[188]   Preparative Example VI: Preparation of 1-cyclopropyl-N-hydroxy-4-[[4-(2-phenoxy-ethoxy)phenyl]sulfonyl]-4-piperidine carboxamide, monohydrochloride
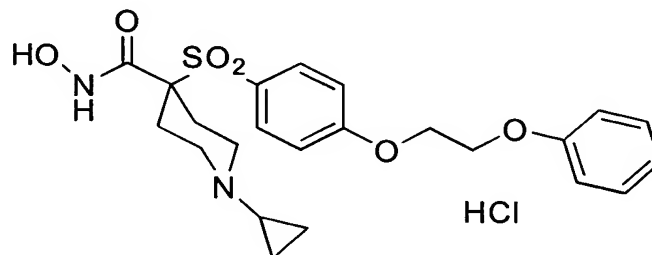
5   **[189]**   Part A: To a solution of the product of Preparative Example II, part E, (14.36 g, 40 mmol) in methanol (50 mL) was added acetic acid (24.5 g, 400 mmol), a portion (about 2 g) of 4-Ångstrom molecular sieves, (1-ethoxycyclopropyl)-oxytrimethyl silane (25.8 mL, 148 mmol) and sodium cyanoborohydride (7.05 g, 112 mmol). The solution was heated at reflux for 8 hr.

10   The precipitated solids were removed by filtration and the filtrate was concentrated *in vacuo*. The residue was diluted with H_2O (400 mL) and extracted with ethyl acetate. The organic layer was washed with saturated NaCl and dried over $MgSO_4$, filtered and concentrated *in vacuo*. The solid was filtered, washed with H_2O/diethyl ether to give the desired cyclopropyl amine {ethyl 4-[(4-fluorophenyl-

15   sulfonyl)]-1-cyclopropyl-4-piperidinecarboxylate} as a white solid (11.83 g, 81.5%).   MS MH^+ calculated for $C_{17}H_{22}NO_4SF$: 356, found : 356.

        [190]   Part B: A solution of the cyclopropyl amine of Part A (2.0 g, 5.6 mmol), ethylene glycol phenyl ether (2.8 mL, 23 mmol), and cesium carbonate (7.3 g, 23 mmol) in DMAC (10 mL) was heat at 125-135°C for 18 hr under an

20   atmosphere of nitrogen. The mixture was concentrated *in vacuo*, diluted with water, and extracted with ethyl acetate. The combined ethyl acetate layers were washed with water and brine, dried over magnesium sulfate, concentrated *in vacuo*, dissolved in diethyl ether, precipitated as the hydrochloride salt, and dried at 40°C in a vacuum oven. The solid was dissolved into a mixture of water, acetonitrile,

25   and ethanol and then the pH was adjusted to 12 with 1N NaOH solution. The

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mixture was concentrated *in vacuo* to remove ethanol and acetonitrile. The solid
was isolated by filtration, washed with water, and dried at 50°C in a vacuum oven
to afford the ether as a white solid (1.8 g, 68%): MS+ calcd. for $C_{25}H_{31}NO_6S$ 474,
found 474. Anal. calcd. for $C_{25}H_{31}NO_6S$: C, 63.40; H, 6.60; N, 2.96; S, 6.77.

5   Found: C, 63.35; H, 6.59; N, 2.99; S, 6.61.

[191]   Part C: A mixture of the ether of part B (1.8 g, 3.7 mmol) and a
50% NaOH aqueous solution (3.0 g, 37 mmol) in THF (32 mL), EtOH (32 mL),
and H_2O (16 mL) was heated at 60°C under a N_2 atmosphere for 24 hr. The
material was concentrated *in vacuo* and triturated with diethyl ether to give a solid.

10   The tan solid was dissolved into a mixture of water, ethanol, and THF, precipitated
by adjusting the pH to 3 with concentrated hydrochloric acid, concentrated *in*
vacuo, triturated with water, and dried at 50°C in a vacuum oven to give a crude
white solid acid (2.3 g).

[192]   A mixture of the crude white solid acid (2.3 g), N-

15   hydroxybenzotriazole (1.9 g, 14 mmol), 4-methylmorpholine (1.6 mL, 14 mmol),
O-tetrahydro-2H-pyran-2-yl-hydroxylamine (1.1 g, 9.4 mmol), and 1-(3-
dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.7 g, 14 mmol) in
DMF (90 mL) was stirred at ambient temperature under a nitrogen atmosphere for
2 days. The mixture was concentrated *in vacuo*, diluted with water, and extracted

20   with ethyl acetate. The organic layer was washed with 1N NaOH solution, water,
and brine, dried over magnesium sulfate, concentrated *in vacuo*, and purification by
flash chromatography (20:80 to 40:60 ethyl acetate/toluene) to afford the protected
hydroxamate as a white solid: (0.43 g, 21%): MS MH+ calcd. for $C_{28}H_{36}N_2O_7S$
545, found 545. Anal. calcd. for $C_{28}H_{36}N_2O_7S$: C, 61.74; H, 6.66; N, 5.14; S, 5.89.

25   Found: C, 61.72; H, 6.75; N, 5.06; S, 5.91.

[193]   Additional compound was isolated by acidifying the aqueous layer
to pH of 3, collecting the solid by filtration, and drying to give a white solid (0.80
g).

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[194] Part D: To an ambient temperature solution of acetyl chloride (0.31 mL, 4.4 mmol) in methanol (11 mL) under a nitrogen atmosphere was added the protected hydroxamate of part C (0.80 g, 1.5 mmol). After stirring for 2.5 hr, the precipitate was collected by filtration, washed with diethyl ether, and dried at 45°C in a vacuum oven to afford the title compound as a white solid (0.58 g, 79%): MS MH+ calcd. for $C_{23}H_{28}N_2O_6S$ 461, found 461. Anal. calcd. for $C_{23}H_{28}N_2O_6S \cdot 1.5HCl$: C, 53.62; H, 5.77; N, 5.44; S, 6.22. Found: C, 53.47; H, 5.79; N, 5.41; S, 6.16.

[195] **Preparative Example VII: Preparation of N-hydroxy-1-(2-methoxyethyl)-4-[[4-[4-(trifluoro-methoxy)phenoxy]phenyl]sulfonyl}-4-piperidinecarboxamide, monohydrochloride**
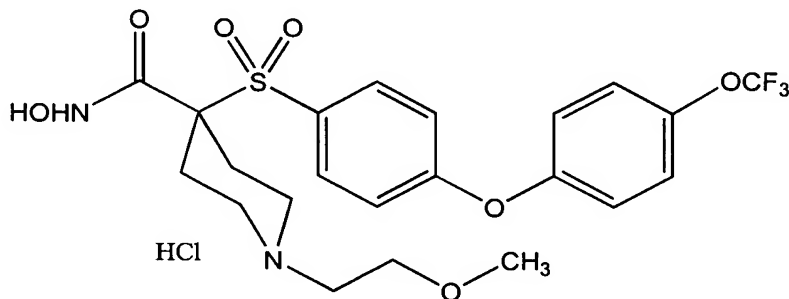
[196] Part A: To a solution of the product of Preparative Example II, Part D (30 g, 161 mmol) in dichloromethane (50 mL) cooled to 0°C was added trifluroacetic acid (25 mL) and the solution was stirred at ambient temperature for 1 hr. Concentration *in vacuo* provided the amine trifluoroacetate salt as a light yellow gel. To the solution of the trifluoroacetate salt and K_2CO_3 (3.6 g, 26 mmol) in N,N-dimethylformamide (50 mL) cooled to 0°C was added 2-bromoethyl methyl ether (19 mL, 201 mmol), and solution was stirred at ambient temperature for 36 hr. Then, N,N-dimethylformamide was evaporated under high vacuum and the residue was diluted with ethyl acetate. The organic layer was washed with water and dried over $MgSO_4$. Concentration *in vacuo* provided the methoxyethyl amine as a light yellow gel (26.03 g, 86.8%).

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[197] Part B: To a solution of methoxyethyl amine (6.0 g, 16.0 mmol) of Part A and powdered K_2CO_3 (4.44 g, 32 mmol) in N,N-dimethylformamide (30 mL) was added 4-(trifluoromethoxy)phenol (5.72 g, 32 mmol) at ambient temperature and the solution was heated to 90°C for 25 hr. The solution was concentrated under high vacuum and the residue was dissolved in ethyl acetate. The organic layer was washed with 1N NaOH, H_2O and dried over $MgSO_4$. Chromatography on silica eluting with ethyl acetate/hexane provided trifluoromethoxy phenoxyphenyl sulfone as a light yellow gel (7.81 g, 91.5%).

[198] Part C: To a solution of trifluoromethoxy phenoxyphenyl sulfone of Part B (7.81 g, 14.7 mmol) in ethanol (14 mL) and tetrahydrofuran (14 mL) was added NaOH (5.88 g, 147 mmol) in H_2O (28 mL) from an addition funnel at ambient temperature. The solution was then heated to 60°C for 18 hr. The solution was concentrated *in vacuo* and diluted with water. The aqueous layer was extracted with ether and acidified to pH = 2. Vacuum filtration of white precipitation provided the acid as a white solid (5.64 g, 73.3%).

[199] Part D: To a solution of the acid of Part C (5.64 g, 10.8 mmol), N-methyl morpholine (4.8 mL, 43.1 mmol), 1-hydroxybenzotriazole (4.38 g, 32.4 mmol) and O-tetrahydropyranyl hydroxyl amine (2.5 g, 21.6 mmol) in N,N-dimethylformamide (50 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (6.2 g, 32.4 mmol), and the solution was stirred at ambient temperature for 24 hr. The solution was concentrated under high vacuum and the residue was dissolved in ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$, H_2O and dried over $MgSO_4$. Concentration *in vacuo* and chromatography on silica eluting with ethyl acetate/hexane provided the tetrahydropyranyl-protected hydroxamate as a white foam (6.65 g, quantitative yield).

[200] Part E: To a solution of 4N HCl in dioxane (28 mL, 110 mmol) was added a solution of the tetrahydropyranyl-protected hydroxamate of Part D (6.65 g, 11.03 mmol) in methanol (3 mL) and dioxane (9 mL) and was stirred at ambient

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temperature for 3 hr. Concentration *in vacuo* and trituration with diethyl ether provided the title compound as a white solid (4.79 g, 78.2%). Analytical calculation for $C_{22}H_{25}N_2O_7SF_3 \cdot HCl \cdot 0.5H_2O$: C, 46.85; H, 4.83; N, 4.97; S, 5.69. Found: C, 46.73; H, 4.57; N, 4.82; S, 5.77.

5

[201]   **Preparative Example VIII: Preparation of N-hydroxy-1-[2-(4-morpholinyl)-ethyl]-4-[[4-[4-(trifluoromethyl)phenoxy]-phenyl]sulfonyl]-4-piperidinecarboxamide, dihydrochloride**
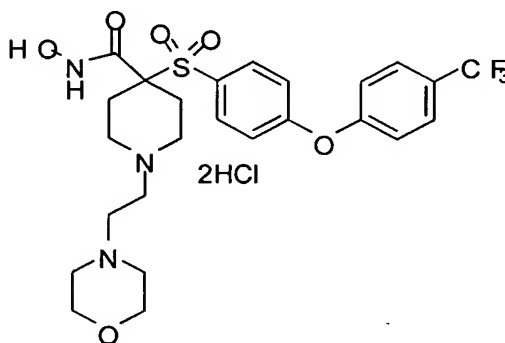
10   [202]   Part A: To a suspension of 4-bromopiperidine hydrobromide (107.0 g, 0.436 mol) in tetrahydrofuran (1 L) was slowly added triethylamine (122 mL, 0.872 mol) followed by di-tert-butyl dicarbonate (100 g, 0.458 mol), which was added in several portions. The resulting mixture was stirred at ambient temperature for 22 hr then filtered and concentrated *in vacuo*. The solids were washed with hexanes and then collected by filtration to give the Boc-piperidine compound as an amber oil (124 g, >100 %).

[203]   Part B: To a solution of 4-fluorophenol (50.0 g, 0.390 mol) in acetone (400 mL), degassed with N_2, was added Cs_2CO_3 (159 g, 0.488 mol). After degassing the resulting mixture with N_2 for 5 min, the Boc-piperidine compound of Part A (85.9 g, 0.325 mol) was added. The resulting mixture was stirred at ambient temperature for 18 hr and then filtered through a pad of Celite®, washing with acetone. The filtrate was concentrated *in vacuo* to provide the sulfide as a tan residue (98.5 g, 97%).

[204]   Part C: To a solution of the sulfide of Part B (8.00 g, 25.7 mmol) in dichloromethane (90 mL) and methanol (15 mL) was added monoperoxyphthalic acid magnesium salt hexahydrate (19.1 g, 38.6 mmol) in two portions. The resulting mixture was stirred at ambient temperature for 1.5 hr and then filtered.

5   The filtrate was washed with saturated $NaHCO_3$ and then with saturated NaCl. The combined aqueous layers were extracted with dichloromethane (100 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated in vacuo. The resulting solids were washed with hexanes then dissolved in dichloromethane and filtered through a pad of Celite®, washing with dichloromethane. The filtrate

10   was concentrated in vacuo and recrystallization from ethyl acetate provided the sulfone as a white crystalline solid (4.45 g, 50%).

[205]   Part D: To a solution of sulfone of Part C (7.00 g, 20.4 mmol) in N,N-dimethylformamide (40 mL) was added Cs_2CO_3 (19.9 g, 61.2 mmol) and α,α,α-trifluoro-p-cresol (3.97 g, 24.5 mmol). The resulting mixture was heated at

15   80°C for 16 hr. After cooling to ambient temperature, the reaction mixture was concentrated in vacuo. The resulting residue was treated with H_2O and the solids were collected by filtration. The solids were then washed with hexanes then methanol to provide the biaryl ether as a tan solid (8.60 g, 87%).

[206]   Part E: To a solution of the biaryl ether of Part D (8.59 g, 17.7

20   mmol) in tetrahydrofuran (100 mL), cooled to 0°C, was slowly added lithium bis(trimethylsilyl)amide (22.0 mL, 1.0M in tetrahydrofuran, 22.0 mmol), at such a rate that the temperature of the reaction never exceeded 1°C. The resulting mixture was stirred at 0°C for 1 hr then a solution of methyl chloroformate (2.05 mL, 26.6 mmol) in tetrahydrofuran (5.0 mL) was slowly added, at such a rate that the

25   temperature of the reaction mixture never exceeded 4°C. After the addition was complete, the mixture was slowly permitted to warm to ambient temperature. Saturated NH_4Cl (50 mL) was added and the tetrahydrofuran was removed in vacuo. Water (50 mL) was added to the residue which was then extracted with ethyl acetate. The combined organic layers were washed with saturated NaCl and

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dried over Na_2SO_4. Recrystallization from methanol provided the methyl ester as a pale yellow crystalline solid (7.66 g, 80%).

[207] Part F: To a solution of the methyl ester of Part E (7.66 g, 14.1 mmol) in dioxane (30 mL) and methanol (10 mL) was added a solution of 4N HCl in dioxane (10 mL, 40 mmol). After stirring at ambient temperature for 2 hr, additional 4N HCl in dioxane (10 mL, 40 mmol) was added. After stirring at ambient temperature for 2.5 hr, the reaction mixture was concentrated *in vacuo* to provide the amine as an off-white solid (6.80 g, >100%).

[208] Part G: To a suspension of the amine of Part F (3.00 g, 6.25 mmol) in acetonitrile (20 mL) was added K_2CO_3 (3.46 g, 25.0 mmol), 4-(2-chloroethyl)morpholine hydrochloride (1.22 g, 6.56 mmol) and a catalytic amount of NaI. The resulting mixture was heated at reflux for 22 hr. After cooling to ambient temperature, the reaction mixture was filtered through a pad of Celite®, washing with ethyl acetate. The filtrate was concentrated *in vacuo* to provide the morpholinyl ethyl amine as a tan solid (3.45 g, >100%).

[209] Part H: To a solution of the morpholinyl ethyl amine of Part G (3.45 g, 6.25 mmol) in tetrahydrofuran (60 mL) was added potassium trimethylsilanolate (1.60 g, 12.50 mmol). After stirring at ambient temperature for 25 hr, H_2O was added. The reaction mixture was then neutralized (pH 7) with 1N HCl. The tetrahydrofuran was removed *in vacuo* and the resulting precipitate was collected by filtration and washed with diethyl ether to provide the amino acid as an off-white solid (2.87 g, 85%).

[210] Part I: To a suspension of the amino acid of Part H (2.87 g, 5.29 mmol) in dichloromethane (25 mL) was added N-methylmorpholine (1.74 mL, 15.9 mmol), O-(tetrahydropuranyl) hydroxylamine (0.682 g, 5.82 mmol) and PyBroP® (2.96 g, 6.35 mmol). After stirring at ambient temperature for 19 hr, additional N-methylmorpholine (0.872 mL, 7.94 mmol), O-(tetrahydropuranyl) hydroxylamine (0.310 g, 2.65 mmol) and PyBroP® (1.48 g, 3.17 mmol) were added. The resulting mixture was stirred at ambient temperature for 3 hr and then

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concentrated *in vacuo*. The residue was partitioned between ethyl acetate and H_2O. The organic layers were washed with saturated NaCl and dried over Na_2SO_4. Chromatography (on silica, methanol/chloroform) provided the protected hydroxamate as an off-white solid (2.62 g, 77%).

5   [211] Part J: To a solution of the protected hydroxamate of Part I (2.62 g, 4.08 mmol) in dioxane (9 mL) and methanol (3 mL) was added a solution of 4N HCl in dioxane (10 mL, 40.0 mmol). The resulting mixture was stirred at ambient temperature for 2 hr and then diethyl ether (20 mL) was added. The resulting solids were collected by filtration to give the title compound as an off-white solid

10   (2.31 g, 90%). MS MH^+ calculated for $C_{25}H_{31}O_6N_3SF_3$: 558, found 558.

[212] **Preparative Example IX: Preparation of 1-cyclopropyl-N-hydroxy-4-[[4-[4-(trifluoromethoxy)phenoxy]-phenyl]sulfonyl]-4-piperidine-carboxamide, monohydrochloride**

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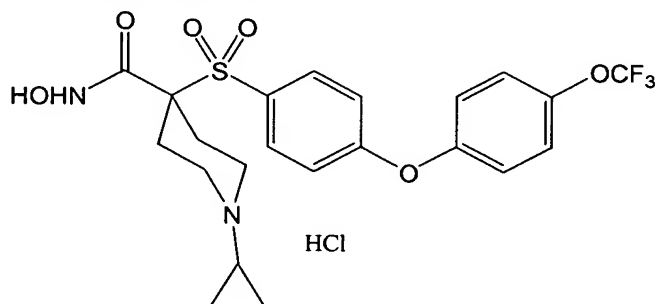
[213] Part A: To a solution of the product of Preparative Example VI, Part A, (6.97 g, 19.6 mmol) in DMF (500 mL) was added K_2CO_3 (3.42 g, 18.0 mmol) and 4-(triflouromethoxy)phenol (3.7 g, 24.8 mmol). The solution was stirred at 90°C for 40 hr. The solution was diluted with H_2O (600 mL) and

20   extracted with ethyl acetate. The organic layer was washed with water, saturated NaCl and dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the desired diaryl ether as an oil (8.5 g, quantitative). HRMS MH^+ calculated for $C_{24}H_{26}NSO_6F_3$: 514.1511. Found 514.1524.

[214]   Part B: To a solution of diaryl ether from Part A (8.4 g, 16.4

mmol) in ethanol (50 mL) and tetrahydrofuran (50 mL) was added a solution of

NaOH (6.54 g, 164 mmol) in water (20 mL) and the solution was heated at 60°C

for 18 hr. The solution was concentrated *in vacuo* to remove most of organic

5   solvents and the aqueous residue was acidified to pH = 4.0. The resulting

precipitate was filtered to give the desired filtered to give the hydrochloride salt as

a white solid (5.01 g, 63%).   HRMS MH$^+$ calculated for $C_{22}H_{22}NSO_6F_3$: 486.1198,

found 486.1200.

[215]   Part C: To a solution of the hydrochloride salt of Part B (5.0 g, 10.3

10   mmol) in DMF (80 mL) were added 1-hydroxybenzotriazole (1.65 g, 12.3 mmol),

N-methyl morpholine (3.4 mL, 30.9 mmol) and O-tetrahydropyranyl

hydroxylamine hydrochloride (1.8 g, 15.4 mmol) followed by 1-3-

(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.60 g, 12.3 mmol).

The solution was stirred at ambient temperature for 42 hr. The solution was

15   diluted with H_2O (400 mL) and extracted with ethyl acetate. The organic layer was

washed with saturated NaCl and dried over $MgSO_4$, filtered and concentrated *in*

vacuo. Chromatography on silica gel, eluting with 30% ethyl acetate/hexane

provided the desired tetrahydropyranyl-protected hydroxamate as a white solid

(5.41 g, 89%).

20   [216]   Part D: To a solution of tetrahydropyranyl-protected hydroxamate

of Part C (5.4 g, 9.2 mmol) in dioxane (80 mL) and methanol (20 mL) was added 4

N HCl/dioxane (50 mL). The reaction was stirred at ambient temperature for 2.5

hr, the solution was concentrated *in vacuo*. Trituration with diethyl ether afforded

the title compound as a white solid (4.02 g, 81%). HRMS MH$^+$ calculated for

25     $C_{22}H_{23}N_2SO_6F_3$: 501.1307, found 501.1324.

[217] Preparative Example X: Preparation of 1-cyclopropyl-N-hydroxy-4-[[4-[4-(trifluoromethyl)phenoxy]phenyl]sulfonyl]-4-piperidinecarboxamide, monohydrochloride
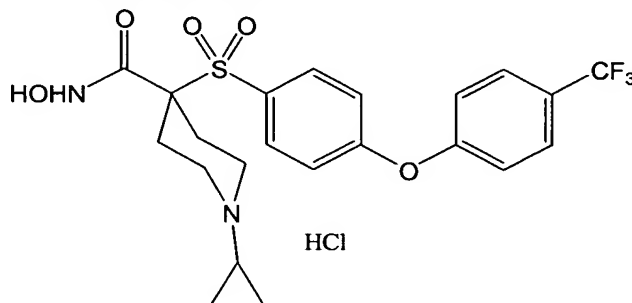
5   **[218]** Part A: To a solution of the product of Preparative Example VI, Part A, (5.96 g, 15.0 mmol) in DMF (100 mL) was added K_2CO_3 (12.34 g, 38.0 mmol) and ☐,☐,☐-trifluoromethyl phenol (3.65 g, 22.5 mmol). The solution was stirred 90°C for 28 hr. The solution was diluted with H_2O (400 mL) and extracted with ethyl acetate. The organic layer was washed with water, saturated NaCl and

10   dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford desired aryl ether as an oil (7.54 g, quantitative)

          [219] Part B: To a solution of aryl ether from Part A (7.54 g, 15.0 mmol) in ethanol (40 mL) and tetrahydrofuran (40 mL) was added a solution of NaOH (6.06 g, 151.0 mmol) in water (20 mL) and the solution was heated at 60°C for 18

15   hr. The solution was concentrated *in vacuo* and the aqueous residue was acidified to pH = 2.0. The resulting precipitate was filtered to give the desired hydrochloride salt as a white solid (7.98 g, quantitative). MS MH^+ calculated for $C_{22}H_{22}NSO_5F_3$: 470, found 470.

          [220] Part C: To a solution of the hydrochloride salt of Part B (7.60 g,

20   15.0 mmol) in DMF (100 mL) were added 1-hydroxybenzotriazole (2.44 g, 18.0 mmol), N-methyl morpholine (3.4 mL, 30.9 mmol) and O-tetrahydropyranyl hydroxyl amine hydrochloride (2.63 g, 22.5 mmol) followed by 1-3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (4.02 g, 21.0 mmol). The solution was stirred at ambient temperature for 96 hr. The solution was

diluted with H_2O (400 mL) and extracted with ethyl acetate. The organic layer was washed with saturated NaCl and dried over $MgSO_4$, filtered and concentrated *in vacuo*. Chromatography on silica eluting with 30% ethyl acetate/hexane provided the desired tetrahydropyranyl-protected hydroxamate as a white solid (5.93g, 69%).

5   [221]   Part D: To a solution of tetrahydropyranyl-protected hydroxamate of Part C (3.8 g, 6.7 mmol) in dioxane (100 mL) was added 4 N HCl/dioxane (30 mL). The reaction was stirred at ambient temperature for 2 hr, then the solution was concentrated *in vacuo*. Trituration with diethyl ether afforded the title compound as a white solid (3.33 g, 96%). MS MH^+ calculated for $C_{22}H_{23}N_2SO_5F_3$:

10   485 , found 485.

[222]   **Preparative Example XI: Preparation of Resin II:**

[223]   **Step 1: Attachment of Compound of Preparative Example IV to Resin I.**

15   [224]   A 500 mL round-bottomed flask was charged with of resin I [Floyd et al., *Tetrahedron Lett.* 1996, *37*, 8045-8048] (8.08 g, 9.7 mmol) and 1-methyl-2-pyrrolidinone (50 mL). A magnetic stirring bar was added, and the resin slurry slowly stirred. A separate solution of the compound of Part D, Preparative Example IV (5.58 g,19.4 mmol) in 1-methyl-2-pyrrolidinone (35 mL) was added to

20   the slurry followed by addition of benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (10.1 g, 19.4 mmol) in one portion. Once the hexafluorophosphate salt had dissolved, 4-methylmorpholine (4.26 mL, 39 mmol) was added dropwise. The reaction slurry was stirred at room temperature for 24 hr, then the resin was collected in a sintered-disc funnel and washed with N,N-

25   dimethylformamide, methanol, methylene chloride and diethyl ether (3x30 mL each solvent). The resin was dried *in vacuo* to yield 10.99 g polymer-bound hydroxymate as a tan polymeric solid. Theoretical loading on polymer was 0.91 mmol/g. FTIR microscopy showed bands at 1693 and 3326 cm^{-1} indicative of the hydroxamate carbonyl and nitrogen-hydrogen stretches, respectively.

[225] Step 2: Preparation of Resin III:

[226] Reaction of Resin II With Nucleophiles

[227]   Resin II (50 mg, 0.046 mmol) was weighed into an 8 mL glass vial,

5   and a 0.5 M solution of a nucleophile in 1-methyl-2-pyrrolidinone (1 mL) was

added to the vessel. In the case of phenol and thiophenol nucleophiles, cesium

carbonate (148 mg, 0.46 mmol) was added, and in the case of substituted

piperazine nucleophiles, potassium carbonate (64 mg, 0.46 mmol) was added. The

vial was capped and heated to 70 to 155°C for 24-48 hr, then cooled to room

10   temperature. The resin was drained and washed with 1-methyl-2-pyrrolidinone, 1-

methyl-2-pyrrolidinone/water (1:1), water, 10% acetic acid/water, methanol, and

methylene chloride (3x3 mL each solvent).

[228] Large Scale Preparation of Resin IIIa:

15   [229]   Resin II (5 g, 0.91 mmol) was weighed into an oven-dried three-

necked round bottom flask fitted with a temperature probe, an overhead stirring

paddle, and a nitrogen inlet. Anhydrous 1-methyl-2-pyrrolidinone (35 mL) was

added to the flask followed by ethyl isonipecotate (7.0 mL, 45.5 mmol). The resin

slurry was stirred slowly with the overhead stirrer, and the mixture was heated to

20   80°C with a heating mantle for 65 hr. The flask was thereafter cooled to room

temperature.

[230]   The resin was collected in a sintered-disk glass funnel and washed

with N,N-dimethylformamide, methanol and methylene chloride (3X30 mL each

solvent). The resin was dried *in vacuo* to provide 5.86 g of resin IIIa as off-white

25   resin beads. The theoretical loading of the polymer was 0.81 mmol/g. TFA

cleavage performed on 50 mg of resin IIIa as described in step 3 yielded 10.4 mg of

off-white solid spectroscopically indistinguishable from a known sample.

[231]   Step 3: Cleavage of Hydroxamic Acids From The Polymer-Support

[232]   Resin III was treated with a trifluoroacetic acid/ water mixture (19:1, 1 mL) for 1 hr at room temperature. During that time, the resin became a deep red color. The resin was then drained and washed with trifluoroacetic acid/water (19:1) and methylene chloride (2x1 mL each solvent), collecting the combined filtrates in a tared vial. The volatiles were removed *in vacuo*, then a toluene/methylene chloride mixture (2 mL each) was added to the residue. The mixture was again concentrated *in vacuo*. The product was characterized by electrospray mass spectroscopy.

[233]   Step 4: Hydrolysis of Polymer-Bound Ester: Preparation of Resin IVa
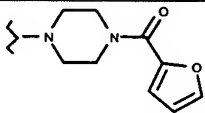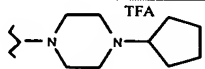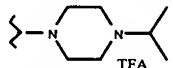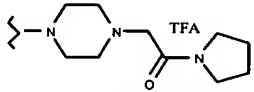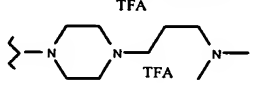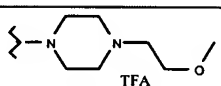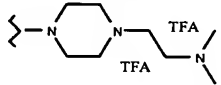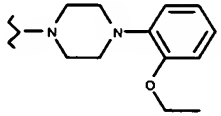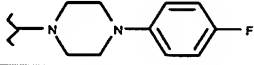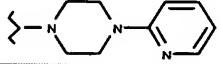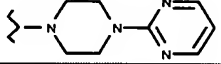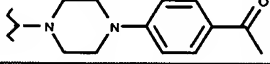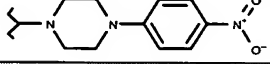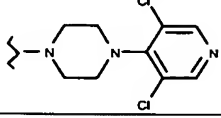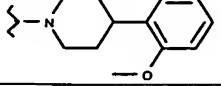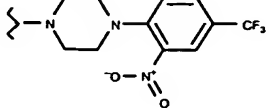
[234]   Resin IIIa (5.8 g, 4.5 mmol) was weighed into a three-necked round bottomed flask fitted with an overhead stirring paddle. 1,4-Dioxane was added to the flask, and the resin slurry was stirred for 15 min. Then, a 4 M solution of KOH (5 mL, 20 mmol) was added, and the mixture was stirred for 44 hr. The resin was thereafter collected in a sintered-disk glass funnel and washed with dioxane/water (9:1), water, 10% acetic acid/water, methanol and methylene chloride (3X30 mL each solvent). The resin was dried *in vacuo* to yield 5.64 g of resin IVa as off-white polymer beads. FTIR microscopy showed bands at 1732 and 1704 cm^{-1} and a broad band from 2500-3500 cm^{-1}. The theoretical loading of the polymer-bound acid was 0.84 mmol/g.

[235]   Examples 1-45
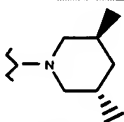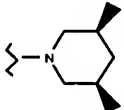
[236]   The following compounds were prepared by parallel synthesis (resin based synthesis, automated synthesis) using parallel synthesis from Resin IVa as described previously in Preparative Example XI the following compounds were prepared:
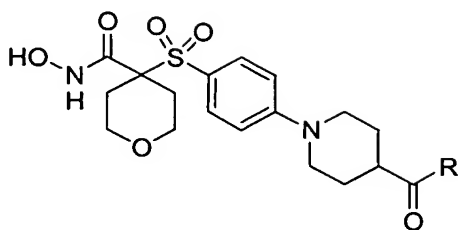
Example	Amine	R	MS (M+H)
1	3,5-Dimethylpiperidine		508
2	1-(1-phenylethyl)-piperazine		585
3	1-(2-phenylethyl)-piperazine		585
4	1-(2-chlorophenyl)-piperazine		591
5	1-(4-methoxyphenyl)-2-methylpiperazine		585
6	1-(5-Chloro-2-methylphenyl)piperazine		605
7	1-(2-methoxyphenyl)-piperazine		587
8	1-Acetylpiperazine		523
9	1-(2,4-Dimethylphenyl)-piperazine		585
10	N-(2-hydroxyethyl)-piperazine		525
11	1-(Ethoxy-carbonylmethyl)-piperazine		567
12	1-(2-Fluorophenyl)-piperazine		575

Example	Amine	R	MS (M+H)
13	1-(2-Furoyl)-piperazine		575
14	1-(Cyclopentyl)-piperazine		549
15	1-(2-Propyl)-piperazine		523
16	N-(2-(1-Piperazino)-acetyl)pyrrolidine		592
17	1-(3-Dimethyl-aminopropyl)-piperazine		566
18	1-(2-Methoxyethyl)-piperazine		539
19	1-(2-Dimethyl-aminoethyl)-piperazine		552
20	1-(2-Ethoxyphenyl)-piperazine		601
21	1-(4-Fluorphenyl)-piperazine		575
22	1-(2-Pyridyl)-piperazine		558
23	2-(1-piperazinyl)-pyrimidine		559
24	4-Piperazino-acetophenone		599
25	1-(4-Nitrophenyl)-piperazine		602
26	1-(3,5-Dichloropyrid-4-yl)piperazine		626
27	4-(2-Methoxyphenyl)-piperidine		586
28	N-[2-Nitro-4-(trifluoromethyl)-phenyl]piperazine		670

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Example	Amine	R	MS (M+H)
29	1-[3-(Trifluormethyl)-pyrid-2-yl]-piperazine		626
30	cis-3,5-Dimethyl-morpholine		510
31	1-(2,4-Difluorphenyl)-piperazine		593
32	1-(4-Pyridyl)-piperazine		558
33	1-(4-Trifluoromethyl-phenyl)-piperazine		625
34	1-Allylpiperazine		521
35	1-(2-Pyrazinyl)-piperazine		559
36	1-[3-Chloro-5-(trifluoromethyl)pyrid-2-yl)]piperazine		660
37	1-(2-(4-Morpholino)-ethyl)piperazine		594
38	3-Chlorophenyl-piperazine		591
39	4-(Hydroxymethyl)-piperidine		510
40	cis-2,6-Dimethyl-piperazine		509
41	3-Methylpiperidine		494
42	1-[4-(Trifluormethyl)-2-pyrimidyl]-piperazine		627
43	1-[4-(Trifluormethyl)-2-pyridyl]-piperazine		626

Example	Amine	R	MS (M+H)
44	3,5-Dimethyl-piperidine		508
45	3,5-Dimethyl-piperidine		508
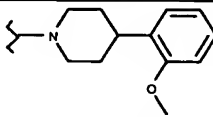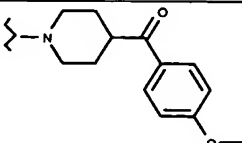
[237] Examples 46-47: Step 12: Further Synthesis of Resin III.

[238] Into a 8 mL glass vial was placed resin II (200 mg, 0.18 mmol) and cesium carbonate (0.98g mg, 3 mmol) (no cesium carbonate used with piperidine and pyrrolidine nucleophiles). One mL of a 1.8 M solution of the amine nucleophile to be reacted in 1-methyl-2-pyrrolidinone (1.8 mmol) was added and the vial was capped and heated to 100°C for 30 hr. Then the vessel was cooled to room temperature, and the resin was drained and washed with 1-methyl-2-pyrrolidinone, 1:1 1-methyl-2-pyrrolidinone/water, water, 1:9 acetic acid/water, methanol and methylene chloride (3X3 mL each solvent).

[239] The following hydroxamic acids were synthesized from Resin III with the indicated amines, followed by release from the polymer using the reaction conditions in Step 3.
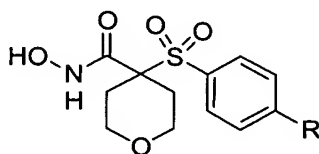
Example	Amine	R	MS (M+H)
46	1-(2-Methoxyphenyl)-piperidine		475
47	4-(4-Methoxybenzoyl)-piperidine		503

74

[240]   Example 48: Preparation of N-hydroxy-4-[[4-(4-methoxyphenoxy)phenyl]sulfonyl]-4-thianecarboxamide
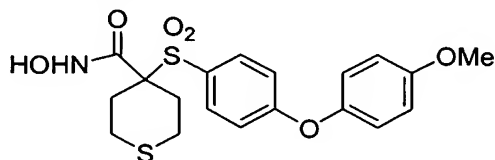
5   [241]   Step 1: Hydrolysis of methyl 4-[[4-(4-methoxyphenoxy)phenyl]sulfonyl]-4-thianecarboxylate. To a solution of methyl 4-[[4-(4-methoxyphenoxy)-phenyl]sulfonyl]-4-thianecarboxylate (10.0 g, 31 mmol) dissolved in tetrahydrofuran (150 mL) was added potassium trimethylsilanolate (12.1 g) and stirred 2 hr. Water was added to the reaction mixture and extracted

10   with ethyl acetate (2x100 mL). The pH value of the aqueous layer was adjusted to 2 with 2M hydrochloric acid and extracted with ethyl acetate (2x100 mL). The latter organics were washed with brine, dried over magnesium sulfate, filtered and the solvent evaporated to afford a pale yellow solid (8.20 g).

       [242]   Step 2: Loading on resin. The compound obtained in step 1 (4.0 g,

15   13.1 mmol) was dissolved in 1-methyl-2-pyrrolidinone (15 mL) and added to a suspension of resin I (6.0 g, 6.6 mmol; Preparative Example XI) in 1-methyl-2-pyrrolidinone (40 mL). To this solution were added pyBOP (6.85 g) and N-methylmorpholine (2.9 mL), and the mixture was stirred with overhead stirring 16 hr. The resin was filtered and washed with dimethylformamide (3x50 mL),

20   methanol (3x50 mL), dichloromethane (3x50 mL) and ether (3x50 mL). The resin was dried *in vacuo* to provide resin MT-III (6.79 g).

       [243]   Step 3: Aryl fluoride displacement of resin MT-III. A suspension of resin MT-III (200 mg, 0.17 mmol), 1-methyl-2-pyrrolidinone (2 mL), cesium carbonate (560 mg) and 4-methoxyphenyl (306 mg) were stirred at 105 °C for 16

25   hr. The reaction mixture was cooled and the resin filtered. The resin was washed with dimethylformamide (3x5 mL), methanol (3x5 mL), 10% aqueous acetic acid (3x5 mL), methanol (3x5 mL) and dichloromethane (3x5 mL). To the resin was

added 95% aqueous trifluoroacetic acid and the reaction mixture was agitated for 1

hr. The resin was drained and washed with dichloromethane (2x1 mL). The

solvent was evaporated. The residue was purified by RPHPLC to provide N-

hydroxy-4-[[4-(4-methoxy-phenoxy)phenyl]sulfonyl]-4-thianecarboxamide (17.9

5   mg) as a pale yellow oil.

[244]   **Examples 49-50**

[245]   The following hydroxamic acids were prepared by the method of

Example 48 using the appropriate amine.
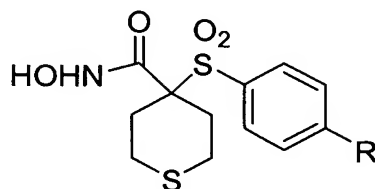
10

Example	R	Amine	MS (ES) m/z
49	4-(4-fluoro-benzoyl) piperidyl	4-(4-fluorobenzoyl)-piperidine	507 (M+H)$^+$
50	4-(2-methoxy-phenyl) piperidyl	4-(2-methoxyphenyl)-piperidine	491 (M+H)$^+$

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[246]   Example 51:   Preparation of N-hydroxy-4-[[4-(4-methoxyphenoxy)phenyl]sulfonyl]-4-thianecarboxamide-1,1-dioxide

[Chemical structure]
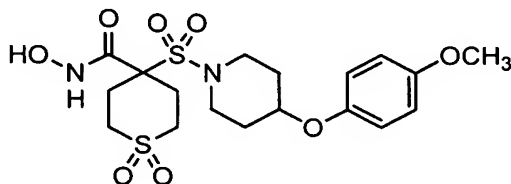
[247]   Step 1:   Oxidation of Resin MT-III. A suspension of resin MT-III
(2.0 g, 1.72 mmol), m-chloroperbenzoic acid (4.37 g) and dichloromethane (25 mL) was stirred at room temperature for 20 hr. The resin was filtered and washed with dichloromethane (3x25 mL), dimethylformamide (3x25 mL), methanol (3x25 mL), 1M aqueous sodium bicarbonate (2x25 mL), methanol (3x25 mL), dichloromethane (3x25 mL) and ether (3x25 mL). The resin was dried *in vacuo* to afford resin MT-IV

(2.16 g).

[248]   Step 2:   Aryl fluoride displacement of resin MT-IV. N-hydroxy-4-[[4-(4-methoxyphenoxy)-phenyl]sulfonyl]-4-thianecarboxamide 1,1-dioxide was prepared by the method of Example 48 using resin MT-IV in the place of resin MT-III. ES (MS) m/z 473 (M+NH$_4$)$^+$.

[249]   Example 52

[250]   The following hydroxamic acid was prepared by the method of Example 51 using 4-(4-fluoro-benzoyl)-piperidine as the amine. MS (ES) m/z 539 (M+H)$^+$.

[Chemical structure]

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[251]   Example 53:   Preparation of N-hydroxy-4-[[4-[4-[(3,5-dimethylpiperidyl)carbonyl]-piperidyl]phenyl]sulfonyl]-4-thianecarboxamide
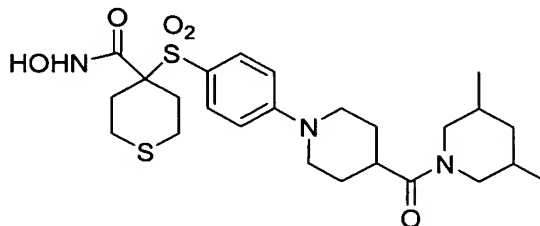
[252]   Step 1: Aryl fluoride displacement of Resin MT-III. To a suspension of resin MT-III (4.06 g, 3.4 mmol) in 1-methyl-2-pyrrolidinone (40 mL) was added ethyl isonipecotate (5.25 mL), and the mixture was heated to 100 °C for 16 hr. The cooled reaction mixture was filtered and the resin was washed with methanol (3x25 mL), dichloromethane (1x10 mL) and ether (3x25 mL). The resin was dried *in vacuo* to afford resin MT-V (4.21 g).

[253]   Step 2: Hydrolysis of resin MT-V. To a suspension of resin MT-V (4.13 g) in tetrahydrofuran (20 mL) was added 4M aqueous potassium hydroxide (10 mL) and stirred at room temperature for 5 days. The resin was filtered and washed with methanol (3x25 mL), dichloromethane (3x25 mL) and ether (3x25 mL). The resin was dried *in vacuo* to afford resin MT-VI.

[254]   Step 3: Conversion to amide. To a suspension of resin MT-VI (268 mg) in 1-methyl-2-pyrrolidinone (2 mL) were added 3,5-dimethyl-piperidine (299 μL), pyBOP (587 mg) and diisopropylethyl amine (393 μL), and mixture was stirred 40 hr. The resin was filtered and washed with dimethylformamide (3x2 mL), methanol (3x2 mL), 10% aqueous acetic acid (3x2 mL), methanol (3x2 mL), dichloromethane (3x2 mL) and glacial acetic acid (1x2 mL). The resin was treated with 95% aqueous trifluoroacetic acid (2 mL) and agitated 1 hr. The resin was washed with dichloromethane (2 mL) and methanol (2 mL). The filtrate was evaporated. The residue was purified by RPHPLC to afford N-hydroxy-4-[[4-[4-[(3,5-dimethylpiperidyl)carbonyl]piperidyl] phenyl]sulfonyl]- 4-thianecarboxamide (7.5 mg) MS (ES) m/z 524 $(M+H)^+$.

[255]   Example 54: Preparation of 1,1-dimethylethyl-3,6-dihydro-4-
[2-(trifluoromethyl)phenyl]-1(2H)-pyridinecarboxylate
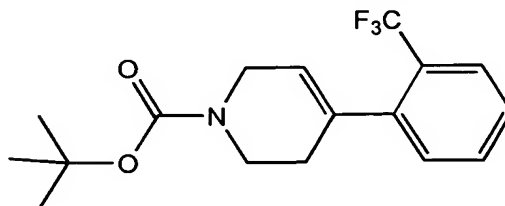
5   [256]   Part A: An oven-dried 1.0 liter flask fitted with a thermometer and
nitrogen inlet was charged with 55 mL of a 2 M solution of lithium
diisopropoylamide in tetrahydrofuran and 50 mL of tetrahydrofuran. The flask was
immersed in a dry ice/acetone bath. When the temperature of the solution was less
than -70°C, a solution of N-t-butoxycarbonylpiperidinone (20.0 g, 0.1 mole) in 100

10   mL tetrahydrofuran was added dropwise, maintaining the temperature less than -
65°C. After complete addition, the flask was stirred with cooling for 20 min. Then
a solution of N-trifluoromethanesulfonimide (38.2 g, 0.107 mole) was added drop-
wise maintaining the temperature less than -65°C. After complete addition, the dry
ice/acetone bath was swapped with an ice/water bath. The reaction was stirred

15   overnight (about 18 hr), slowly warming to room temperature. After 16 hr, the
solvent was removed *in vacuo*, and the residue was purified by column
chromatography on neutral alumina, yielding 26.53 g of product as a yellow oil.
Electrospray mass spectroscopy showed m/z 332 (M+H).

          [257]   Part B: A three-necked 15 mL round-bottom flask was charged

20   with the product from Part A (6 g, 18.1 mmol), o-trifluorobenzeneboronic acid
(4.94 g, 26 mmol), lithium chloride (2.34 g, 55 mmol), 2 M sodium carbonate (26
mL, 52 mmol) and ethylene glycol dimethyl ether (60 mL). Nitrogen was bubbled
through the solution for 10 min, then palladium tetrakistriphenylphosphine (1.06 g,
0.92 mmol) was added. The mixture was heated to reflux for 1.5 hr, then cooled to

25   room temperature. The solvent was removed *in vacuo*, then the residue was
partitioned between 100 mL of methylene chloride and 100 mL of 2 M sodium

carbonate with 3 mL concentrated ammonium hydroxide. The aqueous layer was

extracted with an additional 100 mL methylene chloride, then the combined

organic layers were dried over magnesium sulfate and concentrated to give 8.42 g

of crude product as a dark brown oil. Purification via flash column

5   chromatography (10% ethyl acetate3/hexanes) yielded 2.76 g of pure product as a

yellow oil. Electrospray mass spectroscopy showed m/z 328 (M+H).

[258]   Example 55: Preparation of 1,2,3,6-tetrahydro-4-[2-trifluoromethyl)phenyl]pyridine
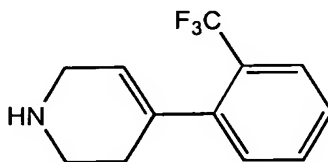
10

[259] The title compound of Example 54 (300 mg, 0.92 mmol) was

dissolved in methylene chloride (5 mL) in a 15 mL round-bottom flask, and 5 mL

of trifluoroacetic acid was added dropwise. After 15 min, the solvent was removed

in vacuo, and the residue partitioned between 20 mL of ethyl acetate and 20 mL of

15   2 M sodium carbonate. The organic layer was washed with additional 2 M sodium

carbonate, dried over magnesium carbonate and concentrated *in vacuo* to yield 195

mg of pure product as a colorless oil. Electrospray mass spectroscopy showed m/z

228 (M+H).

20   **[260]   Example 56: Preparation of 4-[2-(trifluoromethyl)phenyl]piperidine**
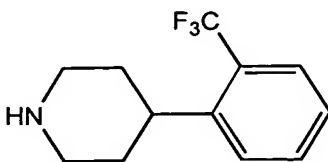
[261]   Part A: A solution of the title compound of Example 54 (2.3 g, 7

mmol) in 20 mL ethanol was added to a hydrogenation flask containing 1 g of 4%

palladium on carbon (0.38 mmol). The mixture was placed under 100 PSI

hydrogen and heated to 50°C for 5 hr. Then the mixture was cooled to room

temperature and filtered through Celite. The filtrate was concentrated *in vacuo* to

give 2.27 g of pure product as a colorless oil. Electrospray mass spectroscopy

5   showed m/z 330 (M+H).

[262]   Part B: The product from Part A above (2.24 g, 6.8 mmol) was

dissolved in 100 mL methylene chloride, and 100 mL of trifluoroacetic acid was

added dropwise. After 15 min, the solvent was removed *in vacuo*, and the residue

partitioned between 100 mL of ethyl acetate and 100 mL of 2 M sodium carbonate.

10   The organic layer was washed with additional 2 M sodium carbonate, dried over

magnesium carbonate and concentrated *in vacuo* to yield 1.12 g of pure product as

a colorless oil. Electrospray mass spectroscopy showed m/z 230 (M+H).

[263]   Example 57:   General Description for Preparation of

15   **Hydroxamic Acids via Aryl Fluoride Displacement with Amines**

[264]   Part A: A 2 dram vial was charged with aryl fluoro compound of

Preparative Example IV (170 mg, 0.44 mmol), 1 ml of 2-methylpyrrolidinone,

cesium carbonate (360 mg, 1.1 mmol) and 0.66 mmol of an amine. A small

magnetic stirring bar was added, then the vial was capped and placed in a Pierce

20   Reacti-therm™ at 115°C. The reaction progress was followed by analytical HPLC.

When the reaction was greater than 90% complete, the vial was cooled to room

temperature. The reaction mixture was diluted with 5 mL of water, then 1.2 mL of

5% hydrogen chloride/water was added dropwise. Then, the entire mixture was

poured onto a column of Celite. The column was washed exhaustively with ethyl

25   acetate (30-40 mL) and the filtrate was collected and concentrated to give the crude

products.

[265]   Part B: The product from above was dissolved in 2 mL 1,4-dioxane

and 2 mL of methanol in a 4 dram vial with a small magnetic stirring bar. A

solution of 4 N hydrogen chloride in 1,4-dioxane was carefully added to the

reaction, and the mixture was stirred for 2 hr. Then the solvent was removed *in vacuo* and the residue purified by preparative reversed-phase HPLC.

[266]   Examples 58-60

5   **[267]**   The following hydroxamic acids were prepared using the method described above in Example 57 with the indicated amine as the starting material.
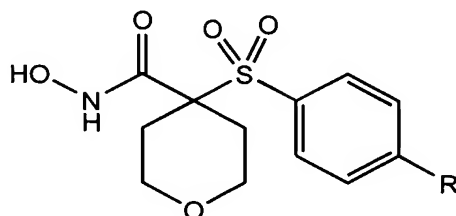
Example	amine	R	m/z from electrospray mass spectroscopy
58	Product of Example 56		513.3 (M+H)
59	Product of Example 55		511.2 (M+H)
60	4-(2-keto-benzimid-azolinyl)-piperidine		501 (M+H)
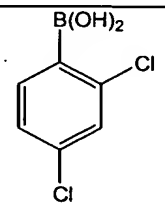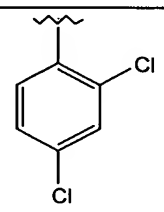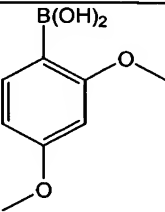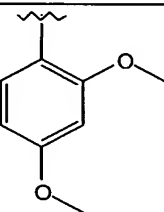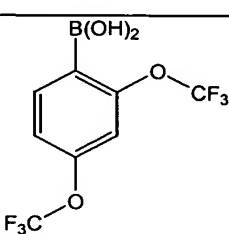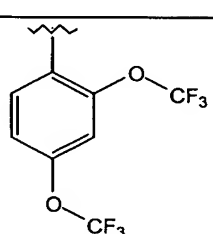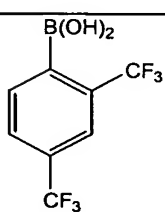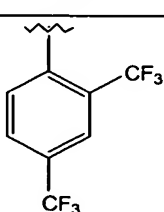
[268] **Examples 61-69**

[269] Using the procedures outlined in Examples 54, 55, and 57 and other methods outlined above, the following analogs are made from the indicated boronic acid:
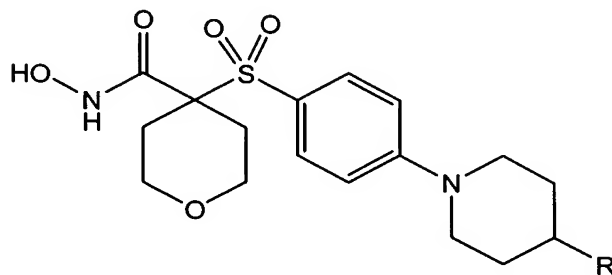
Example	Boronic acid	R
61		
62		
63		
64		
65		

Example	Boronic acid	R
66		
67		
68		
69		

[270] Example 70: Preparation of 4-[[4-[4-[(3,5-dimethyl-1-piperidinyl)carbonyl]-1-piperidinyl]-phenyl]sulfonyl]-N-hydroxy-1-(2-methoxyethyl)-4-piperidinecarboxamide, monohydrochloride

5
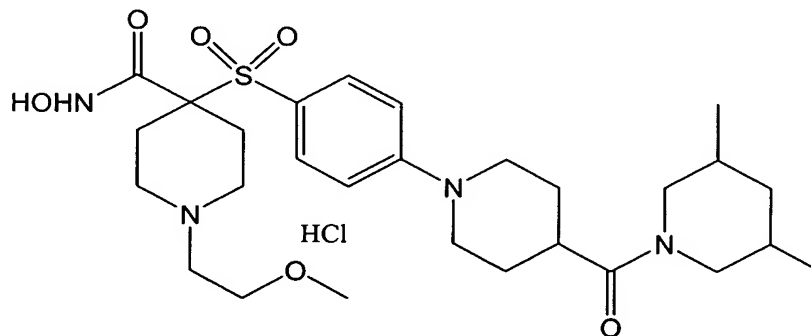
[271]   Part A: To a solution of isonipecotic acid (5.8 g, 44.9 mmol) in water (200 mL) was added sodium carbonate (4.62 g, 44.9 mmol) followed by the drop-wise addition of di-tert-butyl-dicarbonate (10.1 g, 46.3 mmol) in dioxane (40 mL). After 4 hr, the solvent was concentrated *in vacuo* and the solution was extracted with ethyl ether. The aqueous layer was acidified with 3N hydrochloric acid to pH=2. The solution was extracted with ethyl ether and the organic layer was washed with saturated aqueous sodium chloride and dried over magnesium sulfate. Concentration *in vacuo* provided N-Boc-isonipecotic acid as a white solid (9.34 g, 90 %).

[272]   Part B: To a solution of the N-Boc-isonipecotic acid of part A (1.0 g, 4.37 mmol) in dichloromethane (10 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (853 mg, 4.45 mmol), 1-hydroxybenzotriazole hydrate (620 mg, 4.59 mmol) 3,5-dimethylpiperdine (0.67 mL, 5.03 mmol) and diisopropylethylamine (1.67 mL, 9.61 mmol) and was stirred for 21 hr. The solution was concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed with 1M hydrochloric acid, saturated sodium bicarbonate and saturated aqueous sodium chloride and dried over sodium sulfate. Concentration *in vacuo* provided the amide as a clear colorless oil (1.21 g, 89 %).

[273]   Part C: To a solution of the amide of part B (1.20 g, 3.84 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (5 mL) and the solution was stirred for 1 hr. Concentration *in vacuo* provided an oil which was added directly to a solution of the compound of Preparative Example VII, Part A (956 mg, 2.56 mmol) in dimethylacetamide (10 mL). Cesium carbonate (2.92 g, 8.96 mmol) was added and the solution was heated to 100°C for 18 hr. The solution was partitioned between ethyl acetate and water and the organic layer was washed with water and saturated sodium chloride and dried over sodium sulfate. Concentration *in vacuo* provided the phenylamine as an oil (1.53 g, 68 %). MS(CI) MH^+ calculated for $C_{30}H_{47}N_3O_6S$: 578, found 578.

[274]   Part D: To a solution of the phenylamine of part C (1.5 g, 2.6

mmol) in ethanol (9 mL) and tetrahydrofuran (9 mL) was added sodium hydroxide

(1.02 g, 26 mmol) in water (5 mL) and the solution was heated to 60°C for 20 hr.

The solution was concentrated and the residue was diluted with water and acidified

5   to pH = 3 with 3N hydrochloric acid. Vacuum filtration provided the acid as a

beige solid (500 mg, 33 %). MS(CI) MH^+ calculated for $C_{28}H_{43}N_3O_6S$: 550, found

550.

[275]   Part E: To a solution of the acid of part D (492 mg, 0.84 mmol) in

N,N-dimethylformamide (10 mL) was added 1-hydroxybenzotriazole hydrate (136

10   mg, 1.01 mmol), 4-methylmorpholine (0.46 mL, 4.20 mmol), and O-

tetrahydropyranyl hydroxylamine (147 mg, 1.26 mmol). After 1 hr, 1-[3-

(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (225 mg, 1.18 mmol)

was added and the solution was stirred for 72 hr at ambient temperature. The

solution was partitioned between ethyl acetate and water. The organic layer was

15   washed with water and saturated sodium chloride and dried over sodium sulfate.

Concentration *in vacuo* provided the protected hydroxamate as an oil (524 mg, 96

%). MS(CI) MH^+ calculated for $C_{33}H_{51}N_4O_7S$: 649, found 649.

[276]   Part F: To a solution of the protected hydroxamate of part E (514

mg, 0.79 mmol) in 1,4-dioxane (10 mL) was added 4M hydrochloric acid in

20   dioxane (10 mL) and the solution was stirred for 1.5 hr. The solution was

concentrated *in vacuo* and trituration (ethyl ether) provided the title compound as a

white solid (360 mg, 76 %). MS(CI) MH^+ calculated for $C_{28}H_{44}N_4O_6S$: 565, found

565. HRMS calculated for $C_{28}H_{44}N_4O_6S$: 565.3060, found 565.3070. Analytical

calculation for $C_{28}H_{44}N_4O_6S$ 2HCl:2H$_2$O: C, 49.92; H, 7.48; N, 8.32; S, 4.76; Cl,

25   10.52. Found: C, 49.41; H, 7.55; N, 7.85; S, 4.53; Cl, 10.78.

[277] **Example 71: Preparation of 4-[[4-[4-[(3,5-dimethyl-1-piperidinyl)carbonyl]-1-piperidinyl]-phenyl]sulfonyl]-N-hydroxy-1-(2-methoxyethyl)-4-piperidinecarboxamide**
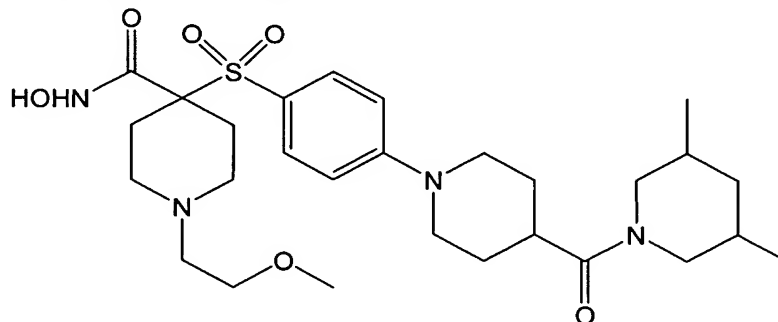
5   A solution of the hydroxamate of Example 70, part F (50 mg, 0.08 mmol) in water (2 mL) was neutralized with saturated sodium bicarbonate. The aqueous solution was extracted with ethyl acetate. Concentration *in vacuo* provided the hydroxamate free base as an orange solid (35 mg, 75%).

10   [278] **Example 72: Preparation of rel-4-[[4-[4-[[(3R,5R)-3,5-dimethyl-1-piperidinyl]carbonyl]-1-piperidinyl]phenyl]sulfonyl]-N-hydroxy-4-piperidinecarboxamide, monohydrochloride**
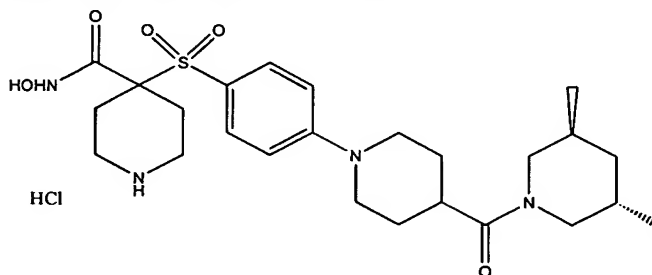
[279] Part A: To a solution of the N-Boc-isonipecotic acid of Example 70,
15   Part A (1.0 g, 4.37 mmol) in dichloromethane (10 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (853 mg, 4.45 mmol), 1-hydroxybenzotriazole hydrate (620 mg, 4.59 mmol) 3,5-dimethylpiperdine (0.67 mL, 5.03 mmol) and diisopropylethylamine (1.67 mL, 9.61 mmol) and was stirred for 21 hr. The solution was concentrated *in vacuo*. The residue was diluted with

ethyl acetate and washed with 1M hydrochloric acid, saturated sodium bicarbonate and saturated sodium chloride and dried over sodium sulfate. Concentration *in vacuo* provided the amide as a clear colorless oil (1.4 g, quantitative yield).

[280] Part B: To a solution of the amide of part A (1.4 g, 4.49 mmol) in dioxane (10 mL) was added 4M hydrochloric acid in dioxane (10 mL) and the solution was stirred for 1 hr. Concentration *in vacuo* provided a solid that was added directly to a solution of the compound of Preparative Example II, Part D, (1.24 mg, 2.99 mmol) in dimethylacetamide (10 mL). Cesium carbonate (3.42 g, 10.5 mmol) was added and the solution was heated to 100°C for 20 hr. The solution was partitioned between ethyl acetate and water and the organic layer was washed with water and saturated sodium chloride and dried over sodium sulfate. Concentration *in vacuo* provided the phenylamine as a yellow solid (1.90 g, quantitative yield). MS(CI) MH$^+$ calculated for $C_{32}H_{49}N_3O_7S$: 620, found 620.

[281] Part C: To a solution of the phenylamine of part B (1.9 g, 3.0 mmol) in ethanol (10 mL) and tetrahydrofuran (10 mL) was added sodium hydroxide (1.2 g, 30 mmol) in water (5 mL) and the solution was heated to 60°C for 20 hr. The solution was concentrated and the residue was diluted with water and acidified to pH = 1 with 3N hydrochloric acid. The solution was extracted with ethyl acetate and washed with 1M hydrochloric acid and saturated sodium chloride and dried over magnesium sulfate. Concentration *in vacuo* provided the acid as a yellow oil (1.9 g, quantitative yield). MS(CI) MH$^+$ calculated for $C_{30}H_{45}N_3O_7S$: 592, found 592.

[282] Part D: To a solution of the acid of part C (1.87 g, 3.00 mmol) in N,N-dimethylformamide (10 mL) was added 1-hydroxybenzotriazole hydrate (486 mg, 3.6 mmol), 4-methylmorpholine (1.65 mL, 15 mmol), and O-tetrahydropyranyl hydroxylamine (526 mg, 4.5 mmol). After 1 hr, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (805 mg, 4.2 mmol) was added and the solution was stirred for 18 hr at ambient temperature. The solution was partitioned between ethyl acetate and water. The organic layer was washed with water and saturated

sodium chloride and dried over sodium sulfate. Chromatography (on silica, ethyl
acetate/hexane) provided the protected hydroxamate as an oil (1.63 g, 79 %).

[283] Part E: To a solution of the protected hydroxamate of part D (1.61
g, 2.33 mmol) in dioxane (10 mL) was added 4M hydrochloric acid in dioxane (10
5   mL) and the solution was stirred for 45 min. The solution was concentrated *in
vacuo* and trituration (ethyl ether) a white solid. Reverse phase chromatography
(on silica, acetonitrile/ water(hydrochloric acid)) produced fractions A, B, C and D.
Concentration *in vacuo* of fraction A provided the title compound as a white solid
(59 mg). MS(CI) MH$^+$ calculated for $C_{25}H_{38}N_4O_5S$: 507, found 507.
10

[284] **Example 73: Preparation of rel-1,1-dimethylethyl 4-[[4-[4-
[[(3R,5R)-3,5-dimethyl-1-piperidinyl]carbonyl]-1-piperidinyl]-
phenyl]sulfonyl]-4-[(hydroxyamino)-carbonyl]-1-piperidinecarboxylate**
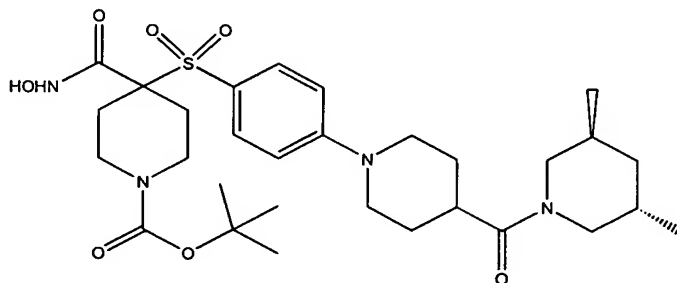
15   From the reverse phase chromatography of Example 72, Part E, fraction C was
concentrated *in vacuo* to provide the title compound as a white solid (49 mg).
MS(CI) MH$^+$ calculated for $C_{30}H_{46}N_4O_7S$: 607, found 607.

[285]   Example 74: Preparation of rel-4-[[4-[4-[[(3R,5S)-3,5-dimethyl-1-piperidinyl]carbonyl]-1-piperidinyl]phenyl]sulfonyl]-N-hydroxy-4-piperidinecarboxamide, monohydrochloride
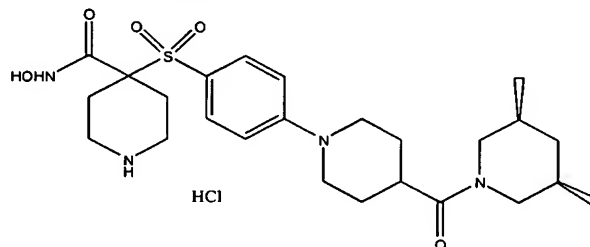
From the reverse phase chromatography of Example 72, Part E, fraction B was concentrated *in vacuo* to provide the title compound as a white solid (198 mg). MS(CI) MH^+ calculated for $C_{25}H_{38}N_4O_5S$: 507, found 507.

[286]   Example 75: Preparation of *rel*-1,1-dimethylethyl 4-[[4-[4-[[(3R,5S)-3,5-dimethyl-1-piperidinyl]carbonyl]-1-piperidinyl]-phenyl]sulfonyl]-4-[(hydroxyamino)-carbonyl]-1-piperidinecarboxylate
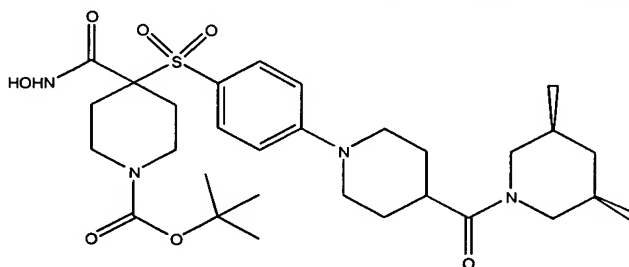
From the reverse phase chromatography of Example 72, Part E, fraction D was concentrated *in vacuo* to provide the title compound as a white solid (242 mg). MS(CI) MH^+ calculated for $C_{30}H_{46}N_4O_7S$: 607, found 607.

[287] Example 76: Preparation of 4-[[4-[4-[(2,3-dihydro-1H-indol-1-yl)carbonyl]-1-piperidinyl]-phenyl]sulfonyl]-N-hydroxy-1-(2-methoxyethyl)-4-piperidinecarboxamide, monohydrate
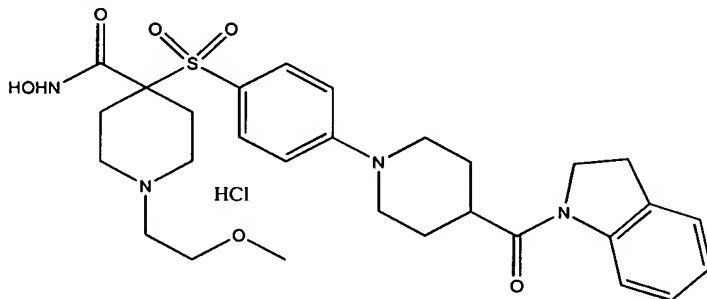
5   [288] Part A: To a solution of the N-Boc-isonipecotic acid of Preparative Example I, Part B (750 mg, 3.27 mmol) in dichloromethane (3 mL) was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (564 mg, 3.21 mmol). The solution was cooled to 0°C and 4-methylmorpholine (0.35 mL, 3.21 mmol) was added. After 2 hr, indoline (0.36 mL, 3.21 mmol) was added and the solution was stirred for 22 hr

10   at ambient temperature. The solution was concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed with 1M hydrochloric acid, saturated sodium bicarbonate and saturated sodium chloride and dried over sodium sulfate. Concentration *in vacuo* provided the amide as a pink solid (940 mg, 89 %).

[289] Part B: To a solution of the amide of part A (935 g, 2.83 mmol) in

15   1,4-dioxane (10 mL) was added 4M hydrochloric acid in dioxane (10 mL) and the solution was stirred for 1 hr. Concentration *in vacuo* provided an oil which was added directly to a solution of the compound of Preparative Example VII, Part A, (705 mg, 1.89 mmol) in dimethylacetamide (10 mL). Cesium carbonate (2.15 g, 6.61 mmol) was added and the solution was heated to 110°C for 18 hr. The

20   solution was partitioned between ethyl acetate and water and the organic layer was washed with water and saturated sodium chloride and dried over sodium sulfate. Concentration *in vacuo* provided the phenylamine as an orange oil (893 mg, 81 %). MS(CI) MH$^+$ calculated for $C_{31}H_{41}N_3O_6S$: 584, found 584.

[290]   Part C: To a solution of the phenylamine of part B (885 g, 1.52 mmol) in ethanol (10 mL) and tetrahydrofuran (10 mL) was added sodium hydroxide (607 mg, 15.2 mmol) in water (5 mL) and the solution was heated to 60°C for 20 hr. The solution was concentrated and the residue was diluted with

5   water and acidified to pH = 1 with 3N hydrochloric acid producing a solid. Vacuum filtration provided the acid as a beige solid (475 g, 53 %). MS(CI) MH^+ calculated for $C_{29}H_{37}N_3O_6S$: 556, found 556.

[291]   Part D: To a solution of the acid of part C (465 g, 0.79 mmol) in N,N-dimethylformamide (10 mL) was added 1-hydroxybenzotriazole hydrate (128

10   mg, 0.95 mmol), 4-methylmorpholine (0.43 mL, 3.95 mmol), and O-tetrahydropyranyl hydroxylamine (139 mg, 1.18 mmol). After 1 hr, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (212 mg, 1.10 mmol) was added and the solution was stirred for 18 hr at ambient temperature. The solution was partitioned between ethyl acetate and water. The organic layer was

15   washed with water and saturated sodium chloride and dried over sodium sulfate. Chromatography (on silica, ethyl acetate/methanol) provided the protected hydroxamate as a yellow oil (305 mg, 60 %). MS(CI) MH^+ calculated for $C_{34}H_{46}N_4O_7S$: 655, found 655.

[292]   Part E: To a solution of the protected hydroxamate of part D (300

20   mg, 0.46 mmol) in dioxane (5 mL) was added 4M hydrochloric acid in dioxane (5 mL) and the solution was stirred for 2 hr. The resulting solid was collected by vacuum filtration. Washing with ethyl ether provided the title compound as a white solid (260 mg, 94 %). MS(CI) MH^+ calculated for $C_{29}H_{34}N_4O_6S$: 571, found 571.

25   [293]   The following compounds were prepared by parallel synthesis (resin based synthesis, automated synthesis) procedures utilizing reactions such as acylation and nucleophilic displacement:

Example 77:
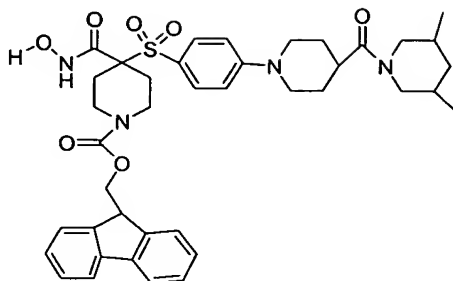
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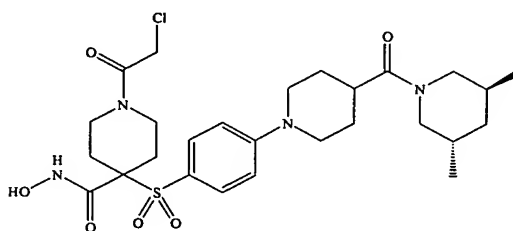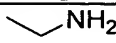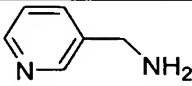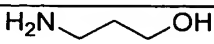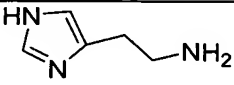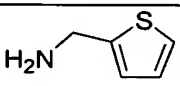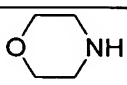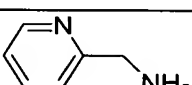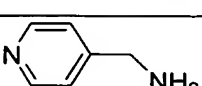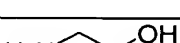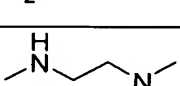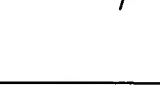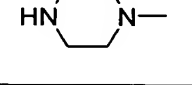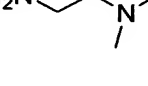
5
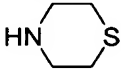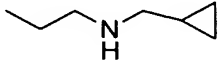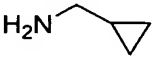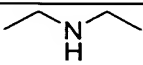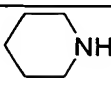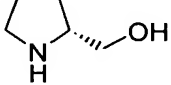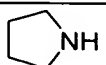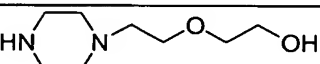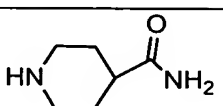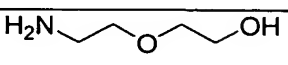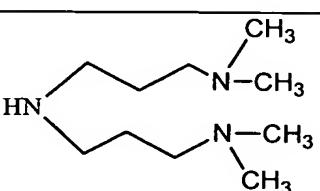
Example 79:

10   **Examples: 80-118**

93
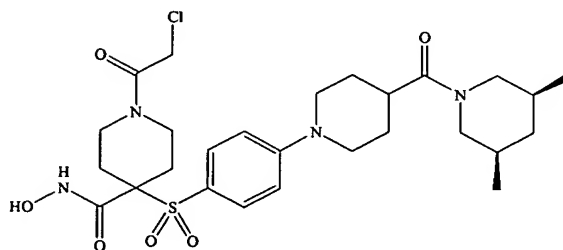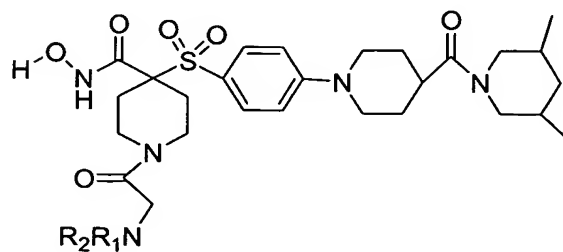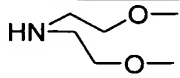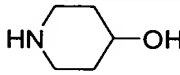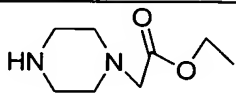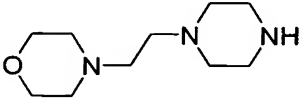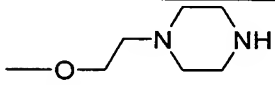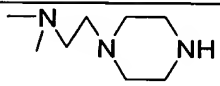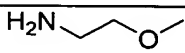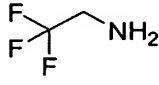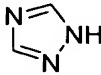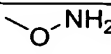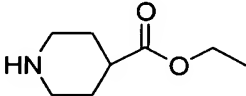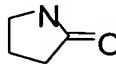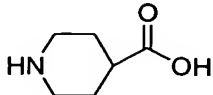
Example	R$_1$R$_2$NH	Amine	MS (ES) m/z
80		Ethyl amine	592 (M+H)
81		3-(Aminomethyl) pyridine	655 (M+H)
82		Imidazole	615 (M+H)
83		3-Amino-1-propanol	622 (M+H)
84		Histamine	658 (M+H)
85		2-Thiophene methyl amine	660 (M+H)
86		Morpholine	634 (M+H)
87		2-(Aminomethyl) pyridine	655 (M+H)
88		4-(Aminomethyl) pyridine	655 (M+H)
89		Ethanolamine	608 (M+H)
90		N,N,N-Trimethyl ethylenediamine	649 (M+H)
91		1-Methylpiperazine	647 (M+H)
92		N,N-Dimethyl ethylenediamine	635 (M+H)
93		Piperazine	633 (M+H)

94

Example	R_1R_2NH	Amine	MS (ES) m/z
94	HN⌒S	Thiomorpholine	650 (M+H)
95	⌒N(H)⌒▷	N-Propylcyclopropne methylamine	660 (M+H)
96	H_2N⌒▷	(Aminomethyl) cyclopropane	618 (M+H)
97	⌒N(H)⌒	Dimethylamine	592 (M+H)
98	⌒N(H)⌒	Diethylamine	620 (M+H)
99	⬡NH	Piperidine	632 (M+H)
100	⬠N(H)⌒OH	(R)-(-)-2-Pyrrolidine methanol	648 (M+H)
101	⬠NH	Pyrrolidine	618 (M+H)
102	HN⬡N⌒O⌒OH	1-(2-(2-Hydroxyethoxy) ethyl)piperazine	721 (M+H)
103	HN⬡⌒C(=O)NH₂	Isonipecotamide	675 (M+H)
104	H_2N⌒⌒O⌒OH	2-(2-Aminoethoxy) ethanol	652 (M+H)
105	HN⌒⌒⌒N(CH₃)—CH₃, ⌒⌒N(—CH₃)CH₃	3,3'-Iminobis(N,N-dimethylpropylamine)	734 (M+H)

Example	R₁R₂NH	Amine	MS (ES) m/z
106		Bis(2-Methoxy ethyl)amine	680 (M+H)
107		4-Hydroxy piperidine	648 (M+H)
108		N-(Carboethoxy methylpiperazine	719 (M+H)
109		1-(2-Morpholinoethyl) piperazine	746 (M+H)
110		1-(2-Methoxyethyl) piperazine	691 (M+H)
111		1-(2-Dimethylaminoethyl) piperazine	704 (M+H)
112		2-Methoxyethylamine	622 (M+H)
113		2,2,2-Trifluoroethyl amine	646 (M+H)
114		1,2,4-Triazole	616 (M+H)
115		Methoxyamine	594 (M+H)
116		Ethyl isonipecotate	704 (M+H)
117		2-Pyrrolidinone	632 (M+H)
118		Isonipecotic acid	676 (M+H)

[294] Example 119 Preparation of:
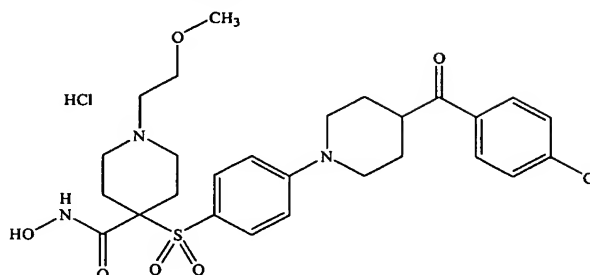
[295] Part A. Preparation of aryl fluoride. To a solution of ethyl 4-[(4-fluorophenyl)sulfonyl]-1-(2-methoxyethyl)-4-piperidinecarboxylate (57 mmol) in dioxane (90mL) and water (45mL) was added LiOH (4.8 g, 3.5 eq). The mixture was stirred at 60°C overnight, cooled to room temperature, and concentrated *in vacuo*. The aqueous layer was treated with concentrated HCl until the pH was approximately 4. The solid was collected and dried. Next, the solid (47.8mmol) in DMF (100 mL) was added NMM (26.2 mL, 239 mmol) and (benzotriazol-1-yl)-N,N,N',N'-bis(tetramethylene)uronium tetrafluoroborate (32.3 g, 62.1 mmol). The mixture was stirred at room temperature for 15 min, and O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (6.71 g, 57.32 mmol) was then added. After 48 hr at room temperature, the mixture was quenched with sat. $NH_4^+Cl^-$, and then extracted with CH_2Cl_2 three times. The combined organic layer was dried and concentrated *in vacuo*. The residue was purified over SiO_2 using hexane/CH_2Cl_2 and then CH_2Cl_2/MeOH to give 20g of protected hydroxyamide as an orange oil.

[296] Part B. Aryl fluoride displacement. A solution of the aryl fluoride from Part A (0.45 mmol), Cs_2CO_3 (1.35 mmol, 3 eq), and 4-(4-chlorobenzoyle)piperidine (Maybridge Chemical Co., England, 0.67 mmol, 1.5 eq) in DMSO (1 mL) was heated to 110°C for 18-48 hr. The mixture was then cooled, dissolved in saturated aq. $NH_4^+Cl^-$ (5 mL), and extracted with dichloromethane (3x3 mL). The combined organic layer was blown down. The crude product was purified by RPHPLC (eluting with 10% to 90% acetonitrile/water), and the pure fractions were combined and concentrated.

[297] Part C. Conversion of the THP hydroxamic acid of Part B to the
hydroxamic acid. The residue from Part B was dissolved in 2 mL of 4M HCl and
1mL of MeOH, stirred at room temperature for 1 h, and then blown down. THEO
M+H= 564.1935; Observed: HI RES M+H= 564.1949;.

5

[298] **Example 120: Preparation of:**
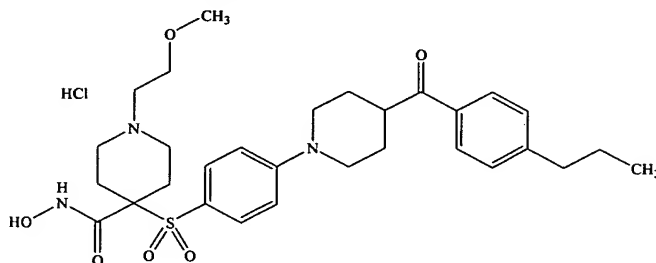
[299] Part A. Preparation of 4-(4-n-propylbenzoyl)piperidine. To a
solution of magnesium (147 mmol, 5 eq) in 40 mL of THF at 0°C was added 1-

10   bromo-4-(n-propyl)benzene (88.24 mmol, 3eq). The solution was allowed to warm
to room temperature over approximately 3 hr. The weinreb amide (29.4 mmol,
1eq) having the following structure:
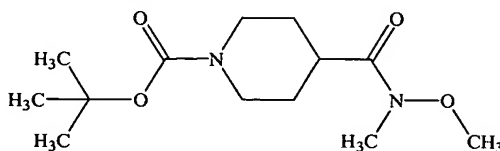
was added, and then the mixture was stirred at room temperature for 18 hr. The

15   mixture was quenched with saturated $NH_4^+Cl^-$, and then extracted with CH_2Cl_2
three times. The combined organic layer was washed with saturated $NH_4^+Cl^-$, dried
over $MgSO_4$, and concentrated *in vacuo*. The residue was purified over 70 g of
SiO_2, eluting with ethylacetate:hexanes (1:10) to ethylacetate:hexanes (1:3). The
piperidine was dissolved in 20 mL of CH_2Cl_2 and 20 mL of trifluoroacetate. The

20   resulting mixture was stirred at room temperature for 1 hr, and then concentrated *in*
vacuo. The residue was treated with 5% NaOH until a solid precipitated out. The
solid was collected and then dissolved in dichloromethane, dried, and concentrated

98

in vacuo. The residue was recrystallized in MeOH/Ether to give 5.07 g of 4-(4-n-propylbenzoyl)piperidine.

[300] Part B. Aryl fluoride displacement. A solution of the aryl fluoride (0.45 mmol., as prepared in Part A of the preceding example), Cs$_2$CO$_3$ (1.35 mmol,

5   3 eq), and the 4-(4-n-propylbenzoyl)piperidine prepared in Part A above (0.65 mmol, 1.5 eq) in DMSO (1 mL) was heated to 110°C for 18-48 hr. The mixture was cooled, dissolved in saturated aqueous NH$_4^+$Cl$^-$ (5 mL), and extracted with dichloromethane (3x3 mL). The combined organic layer was blown down. The crude product was purified by RPHPLC (eluting with 10% to 90%

10   acetonitrile/water), and the pure fractions were combined and concentrated.

[301] Part C. Conversion of the THP hydroxamic acid of Part B to the hydroxamic acid. The residue from Part C was dissolved in 2 mL of 4M HCl and 1mL of MeOH, stirred at room temperature for 1 h, and then blown down. Theo: M+H= 572.2794; Observed: Hi Res M+H= 572.2755;

15

[302] Example 121: Preparation of:

[303] Part A:

weinreb amide   I
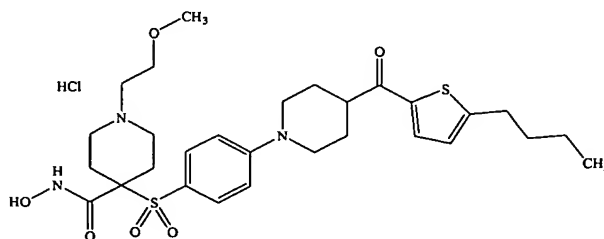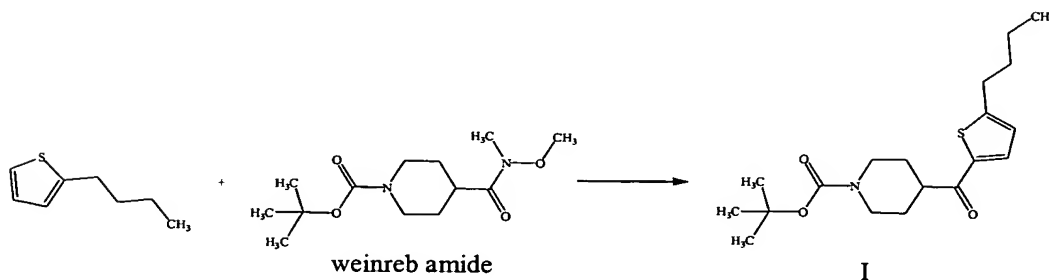
20   To a solution of n-butylthiophene (Lancaster, 5.0 g, MW 140.26, 1.1 eq) in tetrahydrofuran (80 ml) at 0°C was dripped in 1.6 M n-butyllithium in hexanes

(Aldrich, 24 ml, 1.2 eq). The mixture stirred at 0°C for 0.5 hr under N₂. The reaction vessel was then cooled to -78°C, and a solution of the weinreb amide (shown in the reaction above) in tetrahydrofuran (30 ml) was slowly added. The dry ice bath was removed, and the reaction was allowed to warm to room

5   temperature. After 3 hr, the conversion was complete. The reaction was quenched with water (50 ml), and the organic layer was removed *in vacuo*. More water (100 ml) was added, and the mixture was extracted with diethylether (3 x 100 ml). The organic layers were washed with water (2x) and brine (1x), dried over Na₂SO₄, and concentrated to afford a brown oil that was chromatographed

10   (ethylacetate:hexanes, 1:9) to afford 7.5 g of a pale yellow solid (67% crude yield). ¹H NMR showed the desired compound.

[304]   Part B:
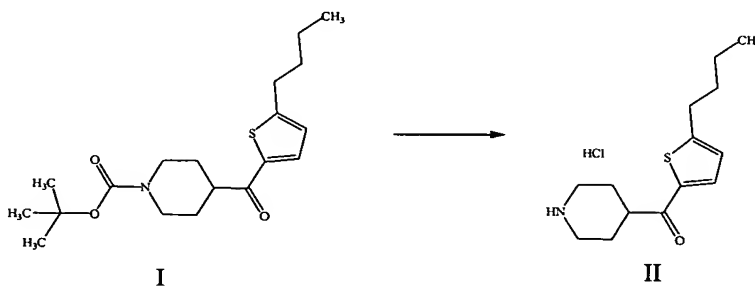
To a solution of Compound I (7.4 g, MW 351.50, 1.0 eq) in acetonitrile (10 ml)

15   was added 4 N HCl in dioxane (Pierce, 40 ml). After 1 hr, the solvent was evaporated, and the residue was slurried in diethylether to afford a white solid that was collected and dried for 5.8 g (97% yield). ¹H NMR showed the desired Compound II.

[305]   Part C:

20

100
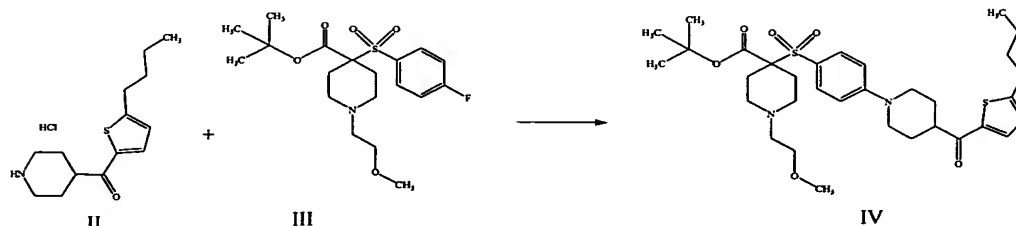
To a solution of Compound II (2.1 g, MW 287.85, 1.5 eq) in dimethylsulfoxide (Aldrich, 15 ml) was added CsCO₃ (Aldrich, 6.4 g, MW 325.8, 4.0 eq). After stirring for 5 min, Compound III (2.0 g, MW 401.49, 1.0 eq) was added, and the mixture was stirred at 90°C for 24 hr. The mixture was then diluted with water (15 ml), and extracted with ethylacetate (3x100 ml). The organic layer was washed with water (1x), washed with brine (2x), dried over Na_2SO_4, and concentrated to a crude brown solid which was recrystallized from hot methanol for 1.83 g of an orange crystalline solid (59% yield). ^{1}H NMR showed the desired Compound IV.

[306] Part D:

IV   V
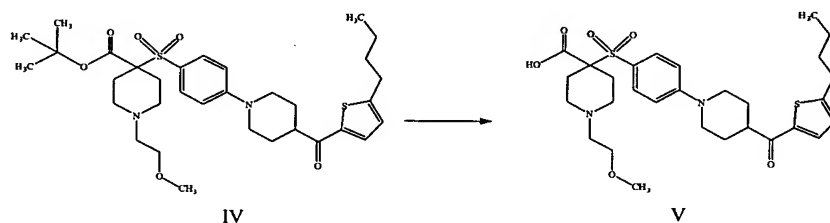
To a solution of Compound IV (1.8 g, MW 632.88, 1.0 eq) in methylene chloride (5 ml) was added trifluoroacetic acid (10 ml). The mixture was stirred for 4 hr at room temperature. The mixture was then concentrated to 1/3 volume, and diethylether was added to afford a solid, which was collected and dried for 1.4 g tan solid (88% yield). ^{1}H NMR and LCMS showed the desired Compound V.

[307] Part E:

V   VI
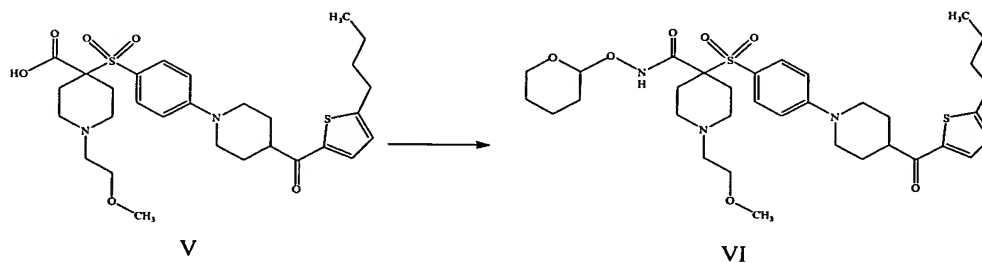
To a solution of Compound V (1.3 g, 2.2 mmol) in N,N-dimethylformamide (8 ml) was added triethylamine (Aldrich, 1.2 ml, 8.8 mmol), followed by N-hydroxybenzotriazole hydrate (Aldrich, 0.6 g, 4.4 mmol), O-tetrahydro-2H-pyran-2-yl)hydroxylamine (0.4 g, 3.3 mmol), and, lastly, 1-(3-dimethylaminopropyl)-3-

ethylcarbodiimide hydrochloride (Sigma, 0.9 g, 4.8 mmol). The mixture was
stirred for 2 days at room temperature. The mixture was diluted with water (10
ml), and extracted with ethylacetate (3x75 ml). The organic layers were combined,
and washed with a saturated sodium bicarbonate solution (1x150 ml) and brine

5   (1x150 ml). The organic layer was then dried over Na_2SO_4, and concentrated to
afford an orange foam that was recrystallized from methanol to afford a pale
yellow solid (1.2 g, 80% yield). ^1H NMR and LCMS showed the desired
Compound VI.

[308]   Part F:

VI   VII

10
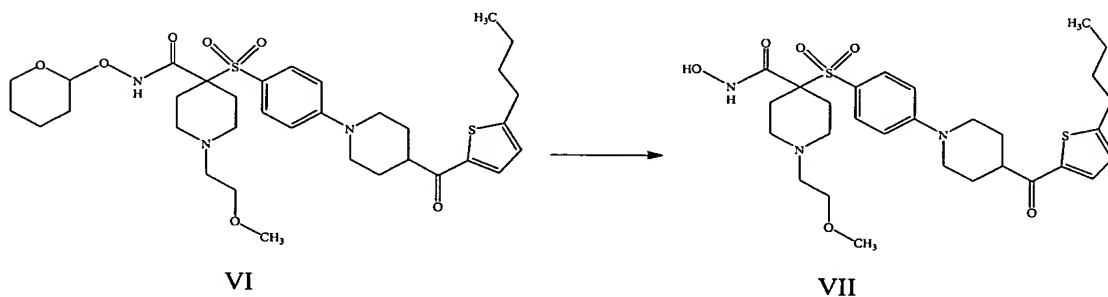
The Compound VI (1.2 g, 1.8 mmol) was treated with methanol (0.5 ml) and 4 N
HCl in dioxane (5 ml) for 1 hr. The solvents were concentrated to 1/3 the volume
via an N_2 stream. Diethylether was then added to the residue to afford a solid that

15   was collected and dried to a white solid (1.0 g, 91% yield). ^1H NMR showed the
desired Compound VII. HRMS confirmed this finding (theo M=H 592.2515,
observed: 592.2498).

[309]   Example 122: Preparation of:

20

[310] Part A:

I
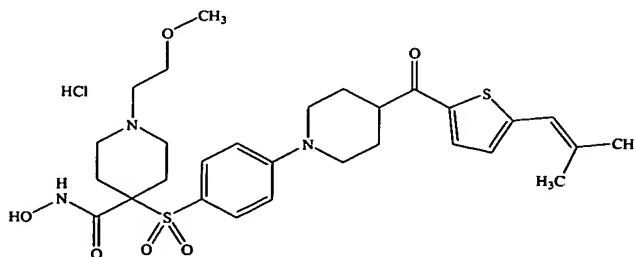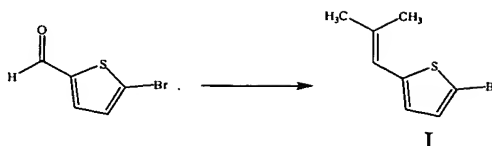
To an ice-cold suspension of isopropylphosphonium iodide (Aldrich, 40.7, MW
432.29, 3.0 eq) at 0°C. in tetrahydrofuran (240 ml) was slowly added n-

5   butyllithium (Aldrich, 1.6 M, 58.9 ml, 3.0 eq). After 1 hr, a solution of 5-bromo-2-
thiophene carboxaldehyde (Aldrich, 6.0 g, MW 191.05, 1.0 eq) in tetrahydrofuran
(60 ml) was added in one shot. The ice bath was removed, and the mixture
warmed to ambient temperature and stirred 2.5 hours. The reaction was quenched
with water (110 ml) followed by 1 N HCl (110 ml). An emulsion developed that

10   was filtered through a coarse frit funnel. The filtrate was separated and the organic
was washed with brine (200 ml), dried over Na_2SO_4, and concentrated to afford a
black oil. Purification on silica gel (ethyl acetate/hexanes) gave 3.4 g of a yellow
oil (50% yield). ^{1}H NMR showed desired Compound I.

[311] Part B:

I   weinreb amide   II

15
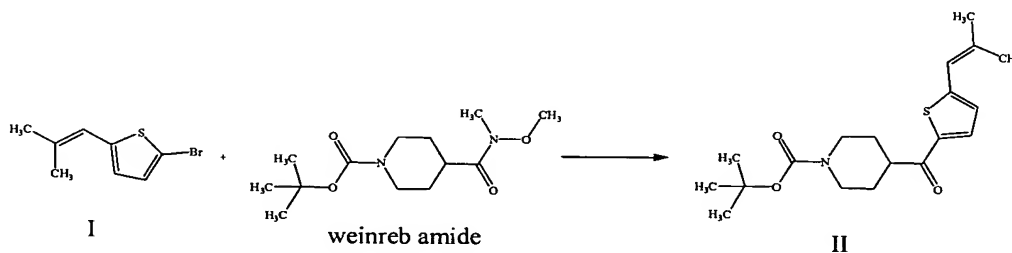
To a solution of Compound I (2.89 g, MW 217.13, 1.5 eq) in tetrahydrofuran (25
ml) at -40°C was dripped 2.0M isopropylmagnesium chloride in tetrahydrofuran
(Aldrich, 6.9 ml, 1.55 eq). The mixture was stirred at -40°C for 1.5 hr under N_2. A
solution of the weinreb amide (shown in the above reaction) in tetrahydrofuran (30

20   ml) was quickly added. The dry ice bath was removed, and the mixture was
allowed to warm to room temperature and stirred overnight. The reaction was
quenched with 1 N HCl (25 ml), followed by water (25 ml). The organic layer was
removed *in vacuo*. The aqueous residue was extracted with diethylether (3x100

ml). The organic layers were washed with water (2x) and brine (1x), dried over
Na_2SO_4, and concentrated to afford a brown oil that was slurried with hexanes. A
solid formed, which was subsequently filtered to afford 1.9 g of gray solid (61%
crude yield). ^1H NMR showed the desired Compound II.

5   [312]   Part C:

II   III
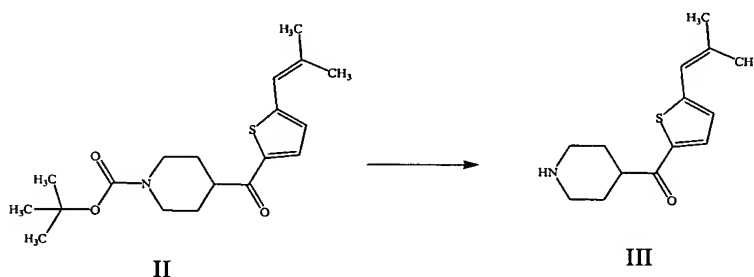
To Compound II (1.9 g, MW 349.49, 1.0 eq) was added 4 N HCl in dioxane
(Pierce, 10 ml). After 1 hr, the solvent was evaporated, and the residue was
slurried in diethylether to afford a gray solid that was collected and dried for 1.4 g

10   (93% yield). ^1H NMR showed the desired Compound III.

[313]   Part D:

III   IV   V
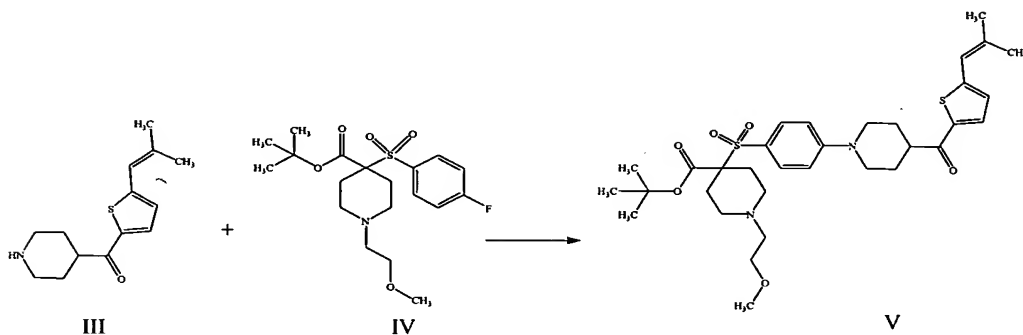
To a solution of Compound III (1.4 g, MW 285.83, 1.5 eq) in dimethylsulfoxide
(Aldrich, 10 ml) was addded $CsCO_3$ (Aldrich, 4.0 g, MW 325.8, 4.0 eq). After 5

15   min, Compound IV (1.3 g, MW 401.49, 1.0 eq) was added, and the reaction was
stirred at 100°C for 24 hr. The mixture was then diluted with water (15 ml), and
extracted with ethylacetate (3x100 ml). The organic layers were washed with
water (1x) and brine (2x), dried over Na_2SO_4, and concentrated for a crude yellow

solid, which was recrystallized from hot methanol for 0.98 g of a yellow crystalline solid (50% yield). LCMS (M+H) showed the desired Compound V.

[314]   Part E:

V

VI

5   To a solution of Compound V (0.98 g, MW 630.86, 1.0 eq) in methylene chloride (4 ml) was added trifluoroacetic acid (4 ml, TFA). The mixture was stirred for 4 hr at room temperature. The mixture was then concentrated to 1/3 volume, and diethylether was added to afford a solid, which was collected and dried for 1.0 g tan solid (93% yield). ^{1}H NMR and LCMS showed the desired Compound VI.

10   [315]   Part F:

VI

VII

To a solution of Compound VI (1.0 g, MW 688.77, 1.0 eq) in N,N-dimethylformamide (5 ml) was added triethylamine (Aldrich, 0.8 ml, MW 101.19, 4.0 eq) followed by N-hydroxybenzotriazole hydrate (Aldrich, 0.38 g, MW 135.13,

15   2.0 eq),)-(tetrahydro-2H-pyran-2-yl), hydroxylamine (0.25 g, MW 117.16, 1.5 eq), and, lastly, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Sigma, 0.59 g, MW 191.76, 2.2 eq). The mixture stirred at ambient temperature for 18 hr.

105

To work up the reaction was diluted with water (10 ml) and extracted with
ethylacetate (3x75 ml). The organics were combined and washed with a saturated
sodium bicarbonate solution (1x150 ml), and brine (1x150 ml). The organic was
then dried over Na$_2$SO$_4$, and concentrated to afford a 0.9 g of a brown oil (96%
5   yield). ^{1}H NMR and LCMS showed the desired Compound VII.

[316]   Part G:
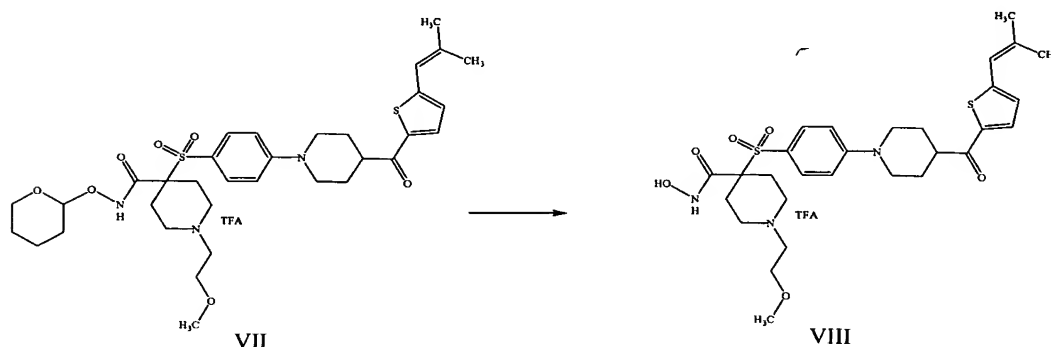
The Compound VII (0.9 g, MW 673.88, 1.0 eq) was treated with methanol (0.5 ml)
and 4 N HCl in dioxane (5 ml) for 1 hr). The solvents were concentrated to 1/3 the
10   volume via an N$_2$ stream. Diethylether was then added to the residue to afford a
solid that was collected and dried for a brown solid (0.32 g, 40% yield). ^{1}H NMR
showed the desired Compound VIII. HRMS confirmed this observation (theo.
M+H 590.2359, observed M+H 590.2364).

15   [317]   Example 123: Preparation of:

106
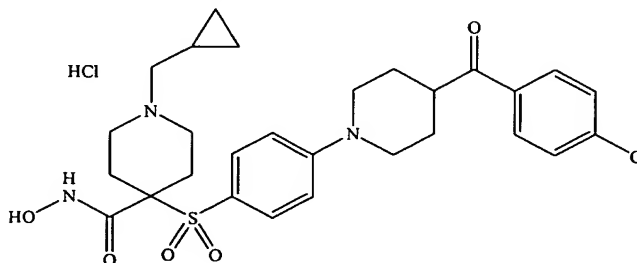
[318] Part A. Preparation of aryl fluoride ester:
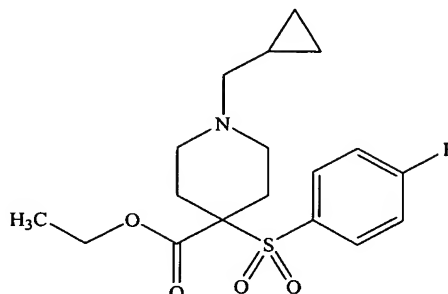
To a solution of molecular slieves (7.5 g), ethyl 5-[(4-fluorophenyl(sulfonyl]-4-piperidinecarboxylate, monohydrate (15g, 42.6 mmol) in methanol (75 mL), and acetic acid (9 mL) was added sodium cyano borohydride (7.23g, 115 mmol). The mixture was stirred at room temperature for 48 hr. The mixture was then quenched with sat. $NH_4{}^+Cl^-$, and extracted with CH_2Cl_2 three times. The combined organic layer was dried and concentrated *in vacuo*. The residue was then recrystallized using ethanol and ether to give 12.1 g (32.7 mmol) of the aryl fluoride ester.

[319] Part B. Aryl fluoride displacement. A solution of the aryl fluoride from Part A (0.45 mmol), Cs_2CO_3 (1.35 mmol, 3 eq), and 4-(4-chlorobenzoyle)piperidine (Maybridge, England, 0.67 mmol, 1.5 eq) in DMSO (1 mL) was heated to 110°C for 18-48h. The mixture was cooled, dissolved in saturated aqueous $NH_4{}^+Cl^-$ (5 mL), and extracted with dichloromethane (3x3 mL). The combined organic layer was blown down, and the crude product was purified by crystallization using ethanol and ether.

[320] Part C. Converting the ethyl ester to the hydroxamic acid. A solution of ethyl ester (5 g) in ethanol (8 mL), tetrahydrofuran (4 mL), and 50% aqueous NaOH (2 mL) was heated to 50°C for approximately 2 hr (additional ethanol and THF can be added if the solid was not completely soluble after 1 hr at 50°C). The residue was neutralized to a pH of 5-6 with aqueous HCl. The aqueous layer was concentrated *in vacuo*, and the resulting solid was washed with acetonitrile and water, and dried under high vacuum. A solution of the acid, NMM (3 eq), EDC (1.4 eq), and HOBT (1.5eq) in DMF (5mL) was heated at 40°C for 2

107

hr. The amine was added, and then stirred at room temperature for 18-48 hr. The

reaction mixture was quenched with saturated aqueous $NH_4^+Cl^-$, and extracted with

dichloromethane. The combined organic layer was concentrated. The THP amide

was purified over SiO_2 using CH_2Cl_2/methanol/triethylamine (the THP amide may

5   alternatively be purified by reverse-phase chromatography). The resulting solid

was then dissolved in 10 mL of 4M HCl and 10 mL of methanol, and stirred at

room temperature until completion (30 min to 120 min). The mixture was then

blown down, and the resulting solid was re-dissolved in methanol and poured into

isopropyl alcohol. The solid was collected and dried. THEO M+H= 560.1986;

10   observed HI RES M+H= 560.1999.

[321]   Example 124: Preparation of:
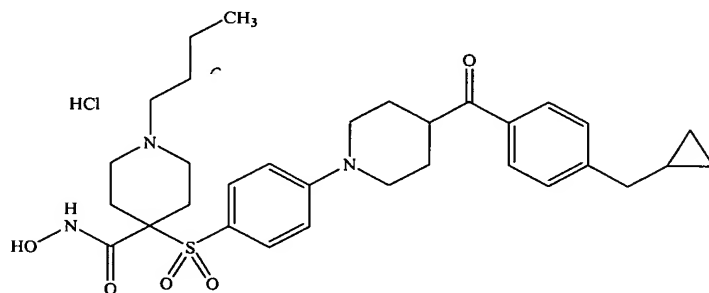
[322]   Part A.   Preparation of 4-(4-metholcyclopropylbenzoyl)piperidine.

15   To a solution of 4-bromophenylcyclopropyl ketone (Acros, 20g, 89 mmol) in THF

(75 mL) was added sodium borohydride (2.25g, 60 mmol) and aluminum

trichloride (3.95g, 30 mmol) in small portions at -5°C. The mixture was allowed to

warm to room temperature for 18 hr, and then stirred an additional 3 hr at 40°C.

The mixture was then cooled, quenched with saturated $NH_4^+Cl^-$, and extracted with

20   CH_2Cl_2 three times. The combined organic layer was dried and concentrated *in*

vacuo. The mixture was chromatographed over 70 g of SiO_2 eluting with

EtOAc:Hexane (0:100 to 10:90) to give 14.55 g (69 mmol) of 4-methyl

cyclopropyl aryl bromide. To a cooled to 0°C solution of the 4-methyl cyclopropyl

aryl bromide (7.75g, 36.7 mmol) in 20 mL of THF was added magnesium (55

108

mmol, 3 eq), followed by dibromoethane (10 uL) in small portions, keeping the mixture cold. The solution was stirred for 3 hr. The weinreb amide described in Example 120 (5g, 18.4 mmol) was added at 0°C, and the mixture was stirred at room temperature for 48 hr. The mixture was then quenched with saturated

5    $NH_4^+Cl^-$, and extracted with CH_2Cl_2 three times. The combined organic layer was dried and concentrated *in vacuo*. The mixture was chromatographed over 70 g of SiO_2 eluting with EtOAc/Hexane (0:100 to 30:70) to give 5.54 g (16 mmol) of the desired BOC-protected piperidine. The BOC-protected piperidine was then dissolved in 20 mL of CH_2Cl_2 and 20 mL of TFA, and stirred at room temperature

10   for 1 hr. The mixture was concentrated *in vacuo*, and the residue was treated with 5% NaOH and water, and then extracted with CH_2Cl_2 three times. The combined organic layer was dried and concentrated *in vacuo* to give 3.47 g (14.3 mmol) of the 4-(4-metholcyclopropylbenzoyl)piperidine.

[323]   Part B. Aryl fluoride displacement. A solution of ethyl 4-[(4-

15   fluorophenyl)sulfonyl]-1-(2-methoxyethyl)-4-piperidinecarboxylate (0.45 mmol), Cs_2CO_3 (1.35 mmol, 3 eq), and 4-(4-metholcyclopropylbenzoyl)piperidine from Part A in DMSO (1 mL) was heated to 110°C for 18-48h. The mixture was cooled, dissolved in saturated aqueous $NH_4^+Cl^-$ (5 mL), and extracted with dichloromethane (3x3 mL). The combined organic layer was blown down, and the

20   crude product was purified by crystallization using ethanol and ether.

[324]   Part C. Converting the ethyl ester to the hydroxamic acid. A solution of ethyl ester (5 g) in ethanol (8 mL), tetrahydrofuran (4 mL), and 50% aqueous NaOH (2 mL) was heated to 50°C for approximately 2 hr (additional ethanol and THF can be added if the solid is not completely soluble after 1 hr at

25   50°C). The residue was neutralized to a pH of 5-6 with aqueous HCl. The aqueous layer was concentrated *in vacuo*, and the resulting solid was washed with acetonitrile and water, and dried under high vacuum. A solution of the acid, NMM (3 eq), EDC (1.4 eq), and HOBT (1.5eq) in DMF (5mL) was heated at 40°C for 2 hr. The amine was added, and then stirred at room temperature for 18-48 hr. The
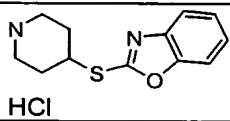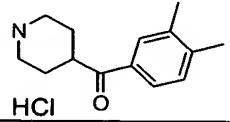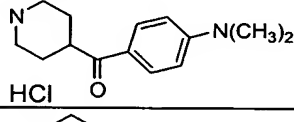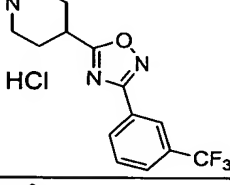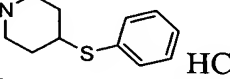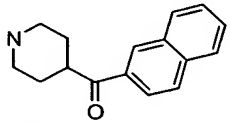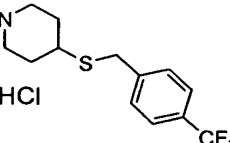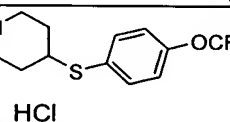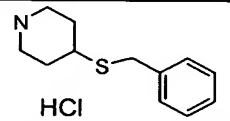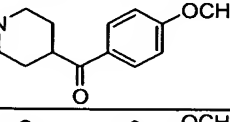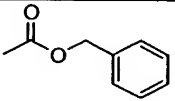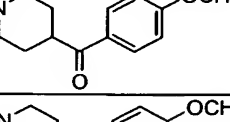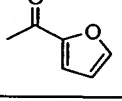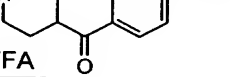
reaction mixture was quenched with saturated aqueous $NH_4^+Cl^-$, and extracted with dichloromethane. The combined organic layer was concentrated. The THP amide was purified over SiO_2 using CH_2Cl_2/methanol/triethylamine (the THP amide may alternatively be purified by reverse-phase chromatography). The resulting solid was then dissolved in 10 mL of 4M HCl and 10 mL of methanol, and stirred at room temperature until completion (30 min to 120 min). The mixture was then blown down, and the resulting solid was re-dissolved in methanol and poured into isopropyl alcohol. The solid was collected and dried. THEO M+H= 584.2794; observed HI RES M+H= 584.2795.
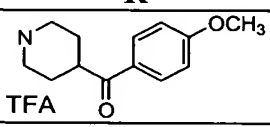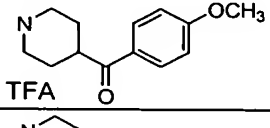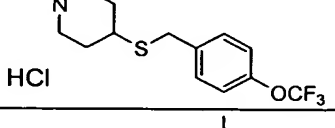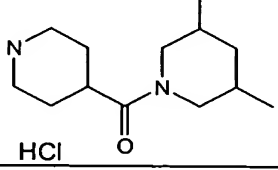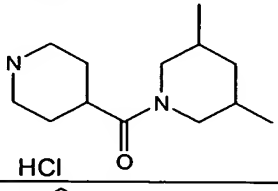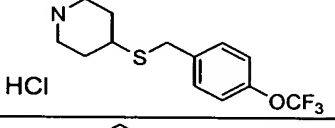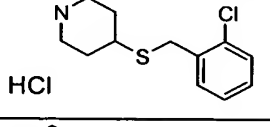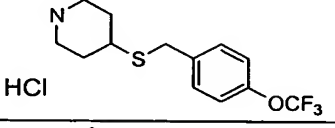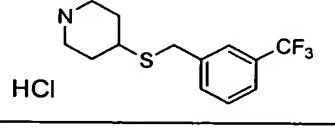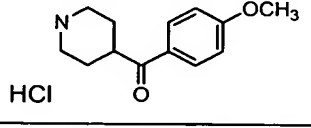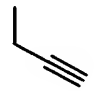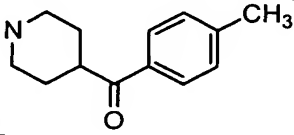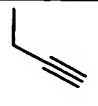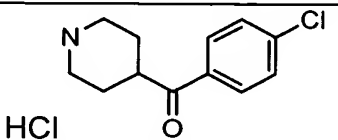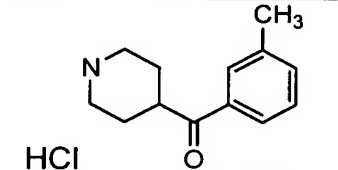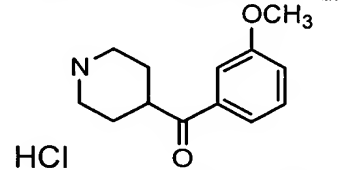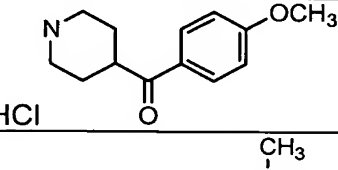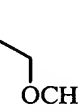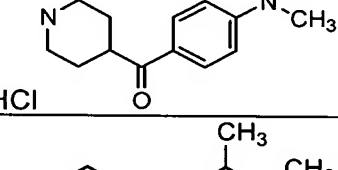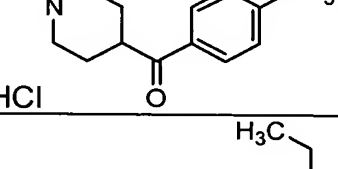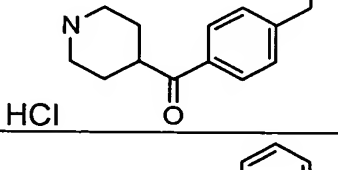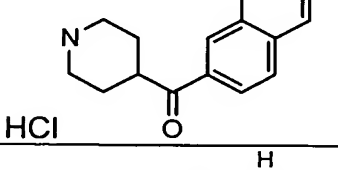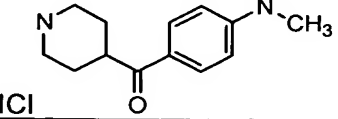
[325] **Examples 125-387**
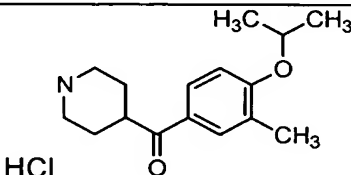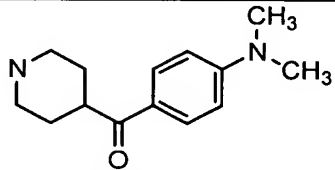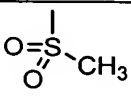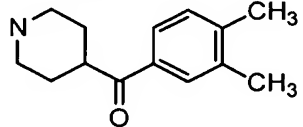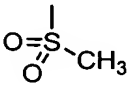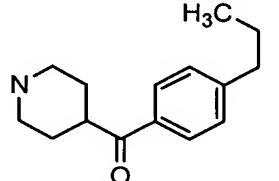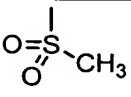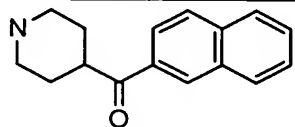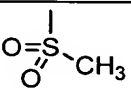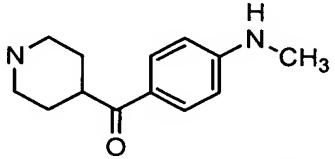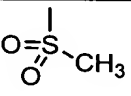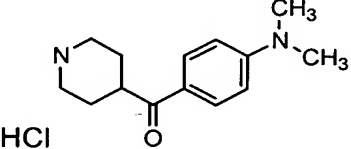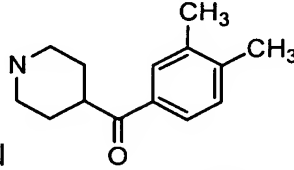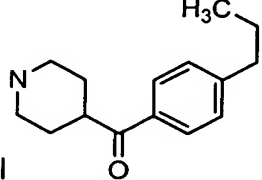
[326] The following compounds were prepared in a manner similar to that used in the preceding examples. In the tables that follow, a generic structure is shown above the table with substituent groups being illustrated in the table along with available mass spectral data.
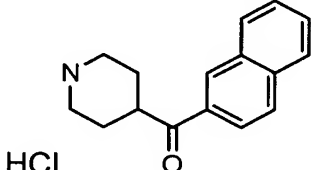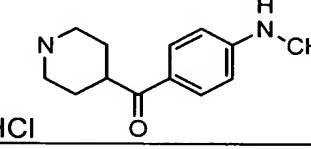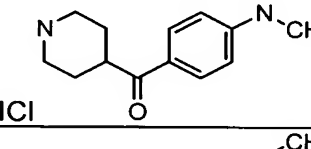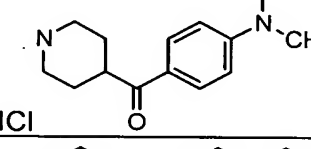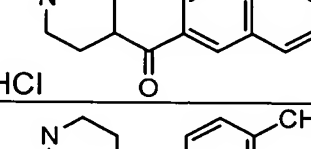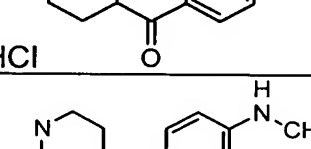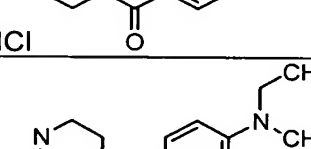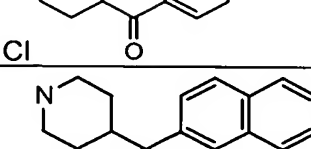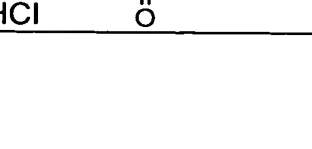
Example	R	K	MS (ES) m/z
125			
126			
127			
128			
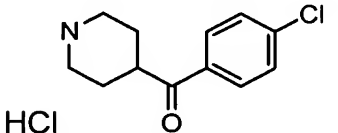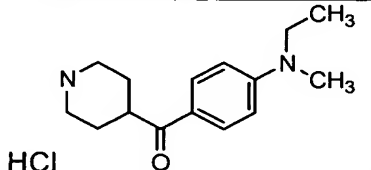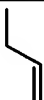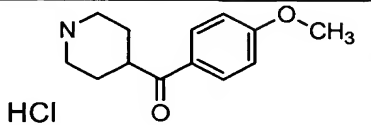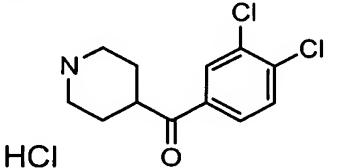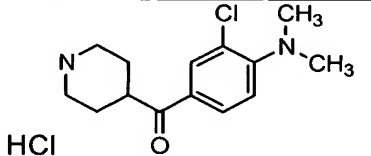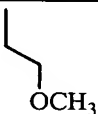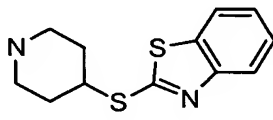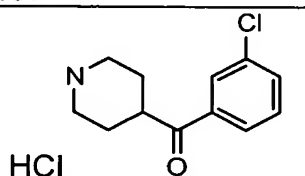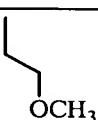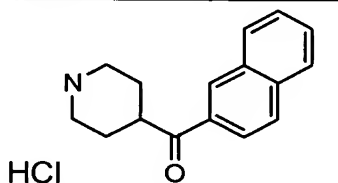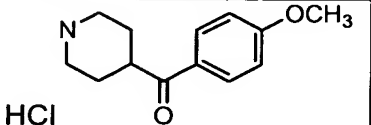
Example	R	K	MS (ES) m/z
129	HCl		
130	HCl		
131	HCl		
132	HCl		
133	HCl		516
134			
135	HCl		
136	HCl		600
137	HCl		530
138			636
139			596
140	TFA	-H	502

111
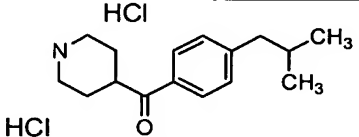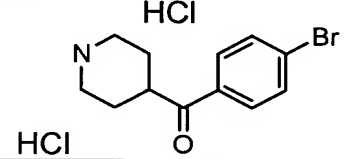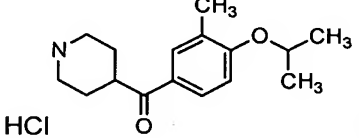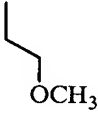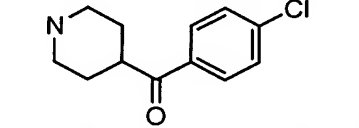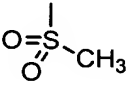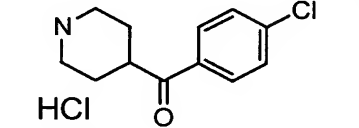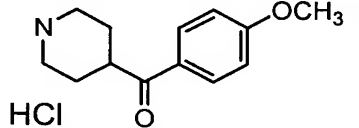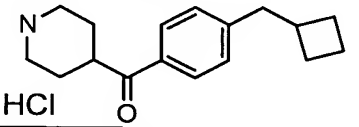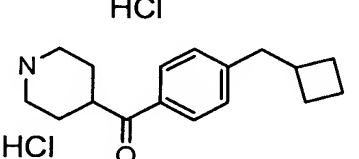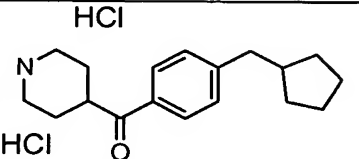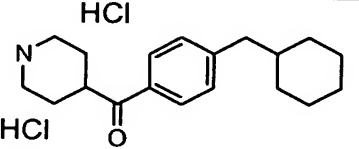
Example	R	K	MS (ES) m/z
141	TFA		579
142	TFA		542
143	HCl		614
144	HCl		585
145	HCl		549
146	HCl	-H	574
147	HCl		564
148	HCl		616
149	HCl		598
151	HCl		540.2160
152	HCl		524.225
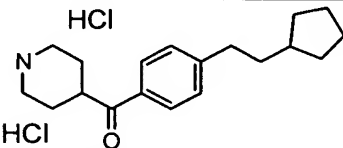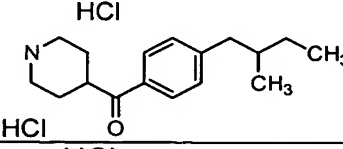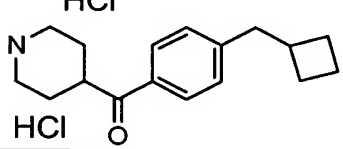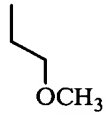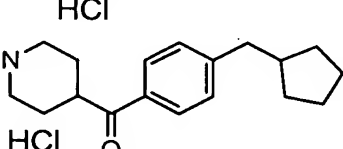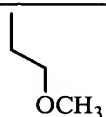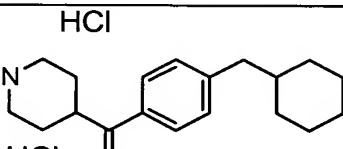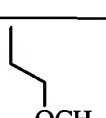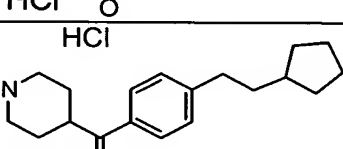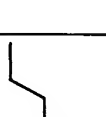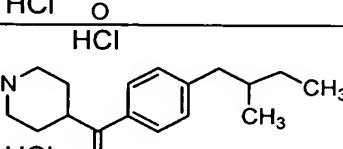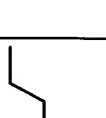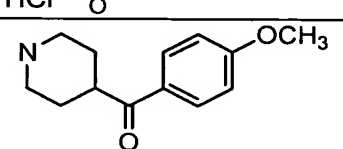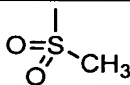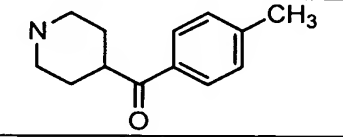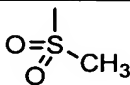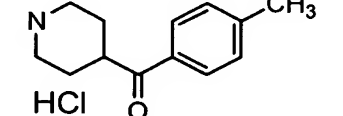
Example	R	K	MS (ES) m/z
153	HCl		544.1673
154	HCl		524.2218
155	HCl		540.2155
156	HCl		560.2387
157	HCl		555.2666
158	HCl		540.2548
159	HCl		554.2698
160	HCl		562.2378
161	HCl		541.2488
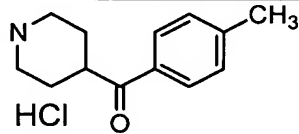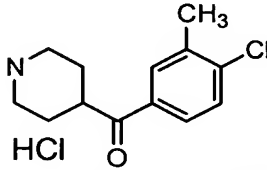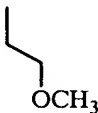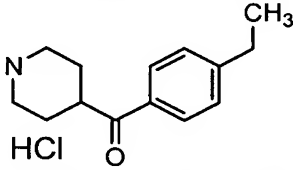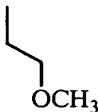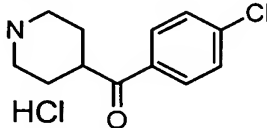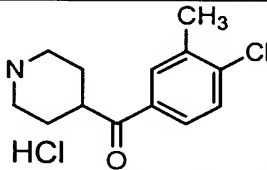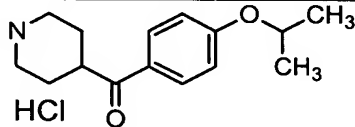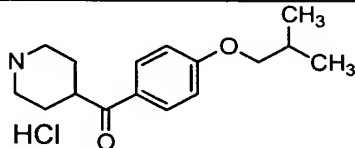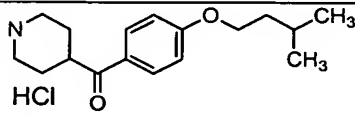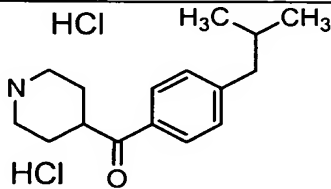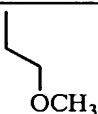
Example	R	K	MS (ES) m/z
162	HCl		
163			593.2131
164			578.1976
165			592.2151
166			600.1865
167			579.1984
168	HCl		543.2647
169	HCl		528.2550
170	HCl		542.2700

114
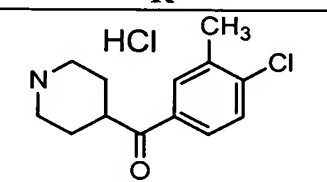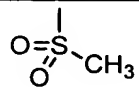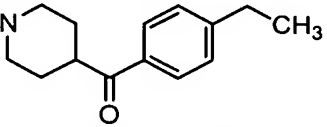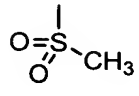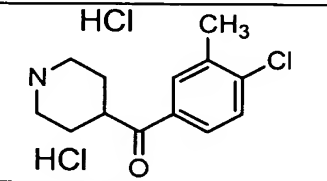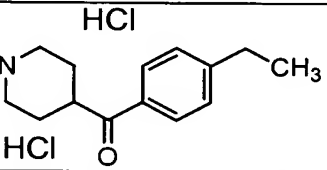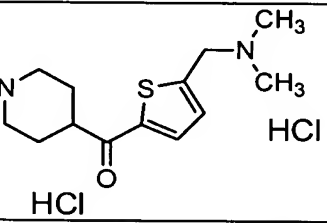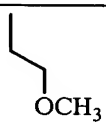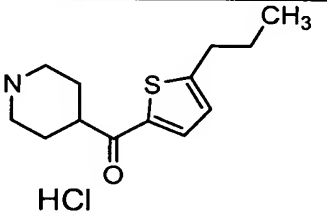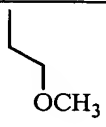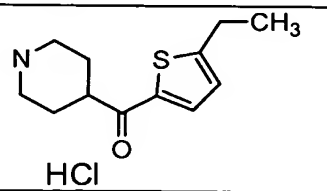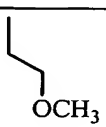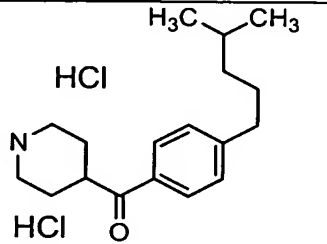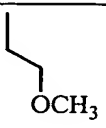
Example	R	K	MS (ES) m/z
171	HCl	CH₃	550.2390⁻
172	HCl	CH₃	529.2505
173	HCl		539.2338
174	HCl		567.2653
175	HCl		560.2249
176	HCl	OCH₃	544.2489
177	HCl	OCH₃	559.2616
178	HCl	OCH₃	587.2933
179	HCl	OCH₃	580.2504

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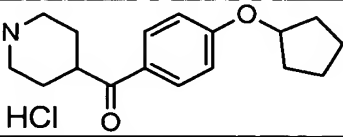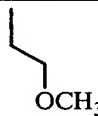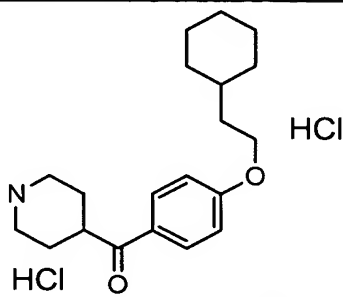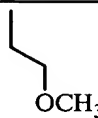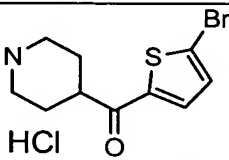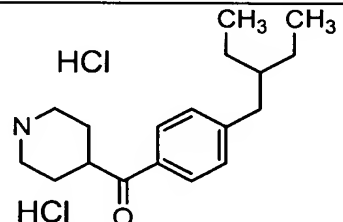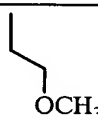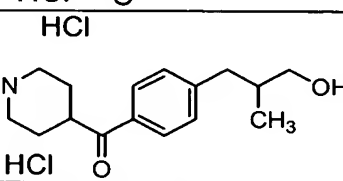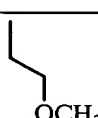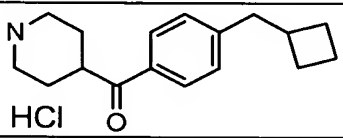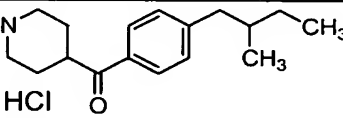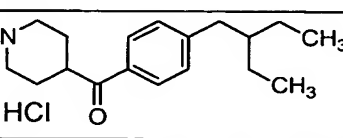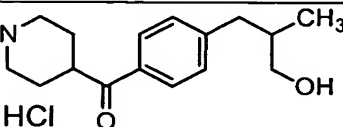
Example	R	K	MS (ES) m/z
180	HCl		546.1840
181	HCl		569.2797
182	HCl		542.2349
183	HCl		578.1288
184	HCl		607.2381
185	HCl		573.1654
186	HCl		564.1965
187	HCl		576.2552
188	HCl		556.2506

116
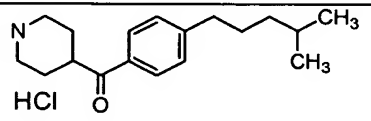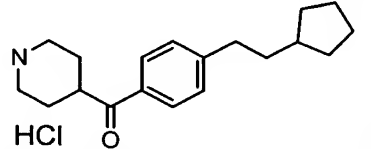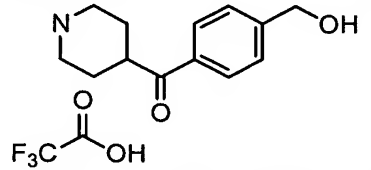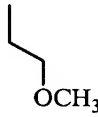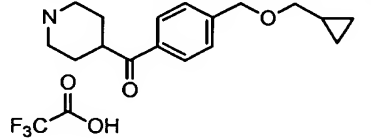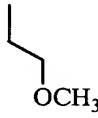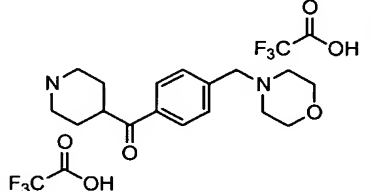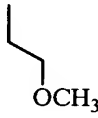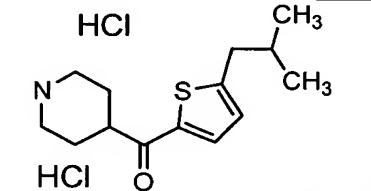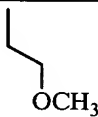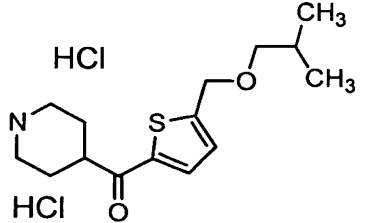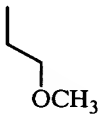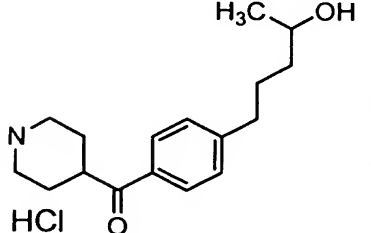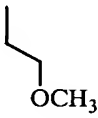
Example	R	K	MS (ES) m/z
189			568.2862
190			590
191			602.2935
192			584.1298
193			534.1860
194			530.2332
195			
196			580.2848
197			594.3011
198			608.3148
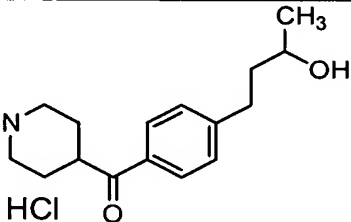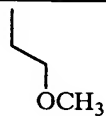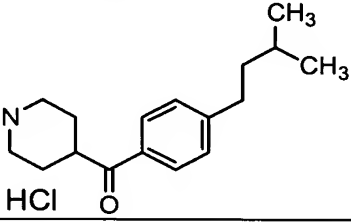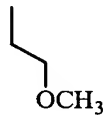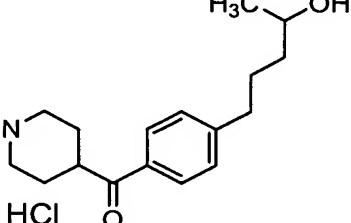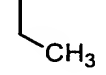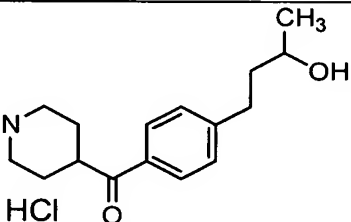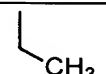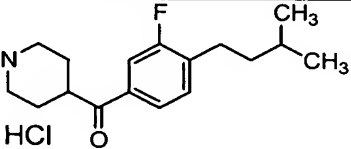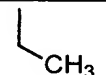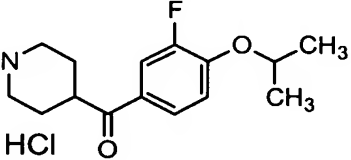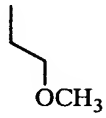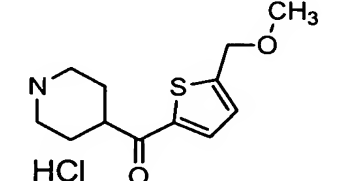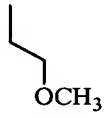
Example	R	K	MS (ES) m/z
199			608.3152
200			582.2997
201			598.296
202			612.3124
203			626.3276
204			626.3268
205			600.3107
206			580.1822
207			546.1850
208			514.2382
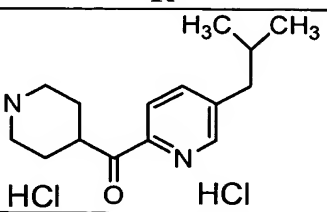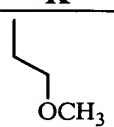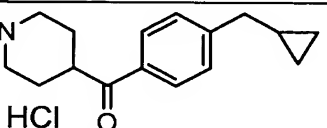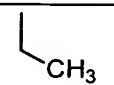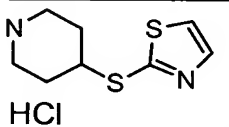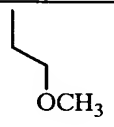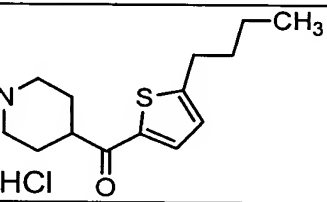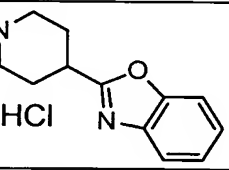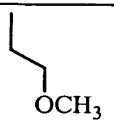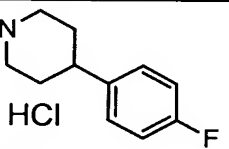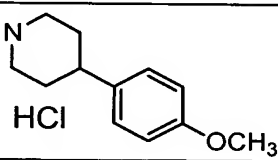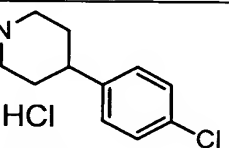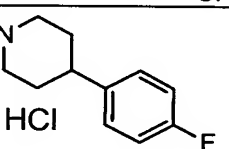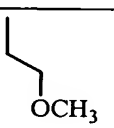
Example	R	K	MS (ES) m/z
210			540.2539
211			578.2106
212			558.2667
213			546.1847
214			560.2012
215			570.2608
216			584.2755
217			598.2953
218			586

119
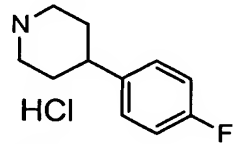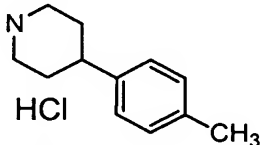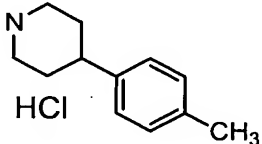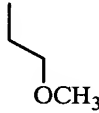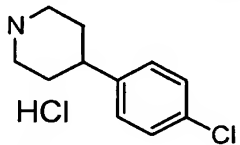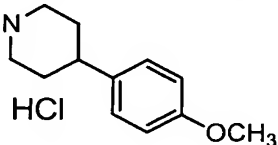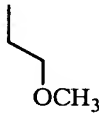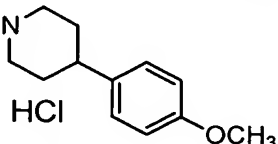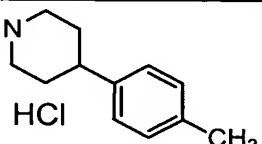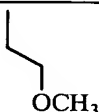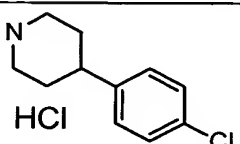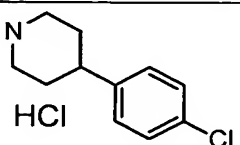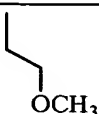
Example	R	K	MS (ES) m/z
219			598.1441
220			578.1966
221			548.1988
222			528.2543
223			593.2431
224			578.2359
225			564.2202
226			614.3261
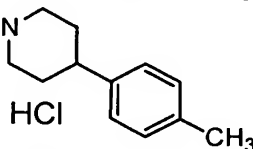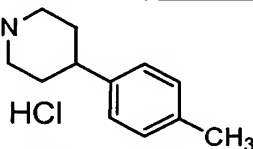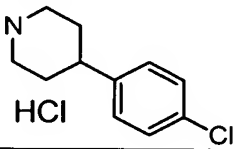
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Example	R	K	MS (ES) m/z
227			614.2932
228			656.3399
229			
230			614.3273
231			602.2901
232			568.2876
233			570.2970
234			584.3166
235			572.2787

Example	R	K	MS (ES) m/z
236			584.3161
237			596.3014
238			560.2437
239			614.2883
240			629.3014
242			
243			622.2636
244			616.3040
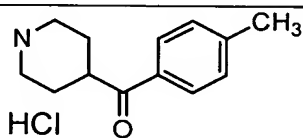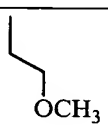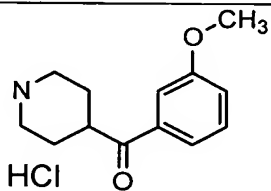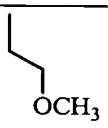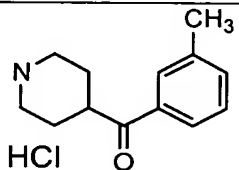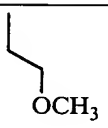
Example	R	K	MS (ES) m/z
245			602.2876
246			600.3109
247			586.2949
248			572.2778
249			570.3007
250			606.2664
252			580.2147

Example	R	K	MS (ES) m/z
253			587.2914
254			554.5692
256			541.1650
257			574.2388
258			543.2291
259			500.2019
260			514.2376
261			516.1723
262			518.2130

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Example	R	K	MS (ES) m/z
263	HCl, piperidine with 4-fluorophenyl	cyclopropyl-ethyl	514.2194
264	HCl, piperidine with 4-methylphenyl (CH₃)	cyclopropyl-ethyl	518.2432
265	HCl, piperidine with 4-methylphenyl (CH₃)	OCH₃ propyl	514.2375
266	HCl, piperidine with 4-chlorophenyl (Cl)	cyclopropyl-ethyl	530.1880
267	HCl, piperidine with 4-methoxyphenyl (OCH₃)	OCH₃ propyl	532.2307
268	HCl, piperidine with 4-methoxyphenyl (OCH₃)	cyclopropyl-ethyl	528.2557
269	HCl, piperidine with 4-methylphenyl (CH₃)	OCH₃ propyl	516.2557
270	HCl, piperidine with 4-chlorophenyl (Cl)	cyclopropyl-methyl	518.1880
271	HCl, piperidine with 4-chlorophenyl (Cl)	OCH₃ propyl	536.1979

Example	R	K	MS (ES) m/z
272	HCl		498.2450
273	HCl		512.2615
274	HCl		532.2061
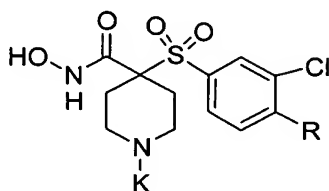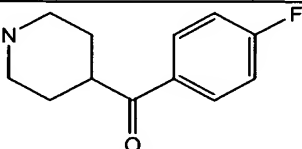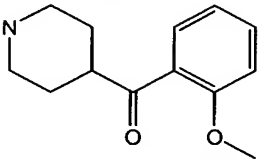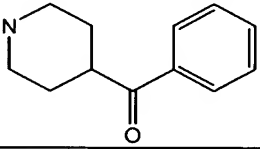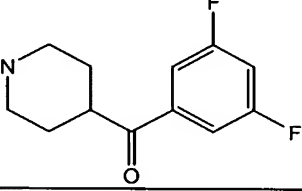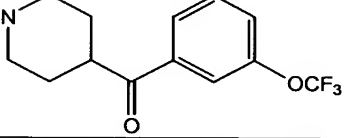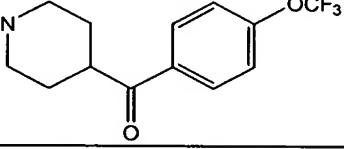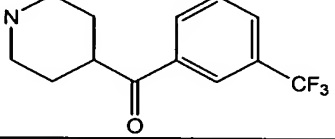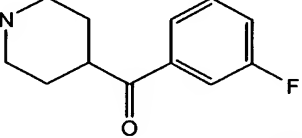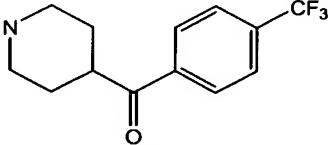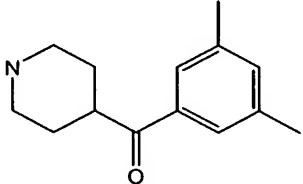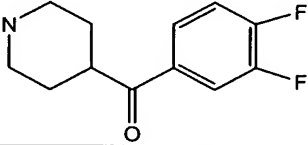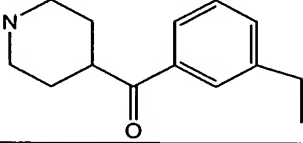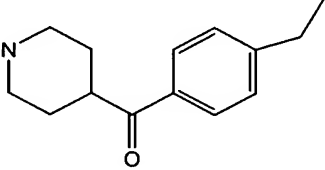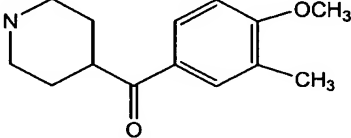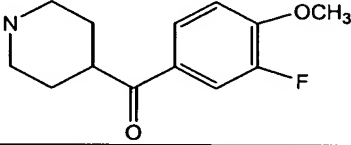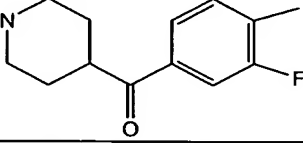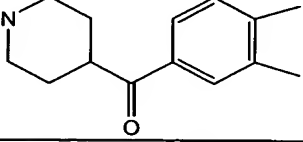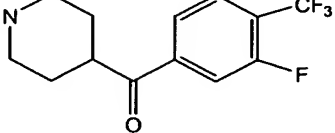
Example	R	K	MS (ES) m/z
275	HCl		578.2068
276	HCl		594.2005
277	HCl		578.2053

126
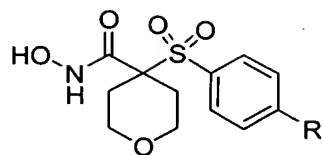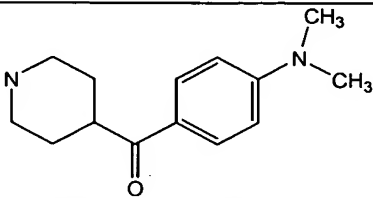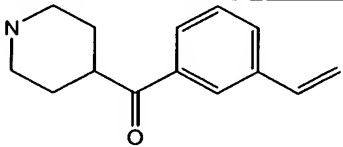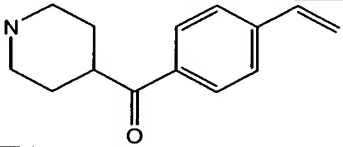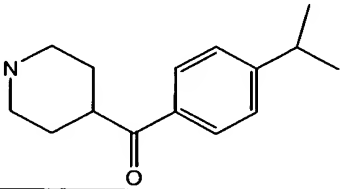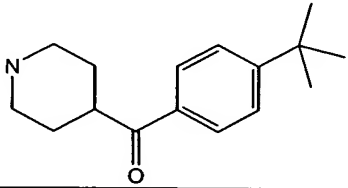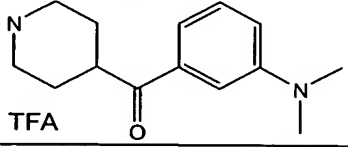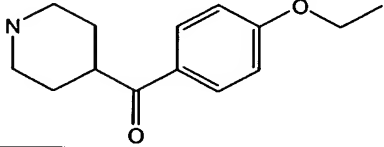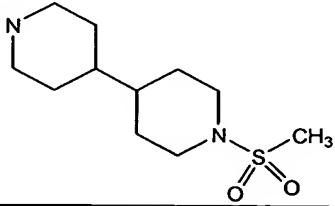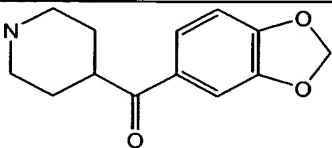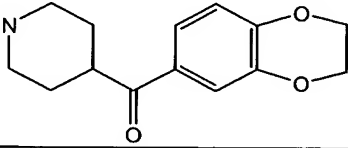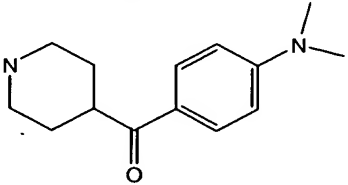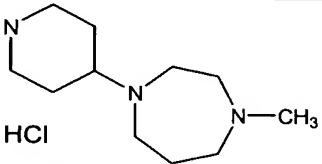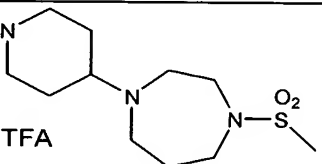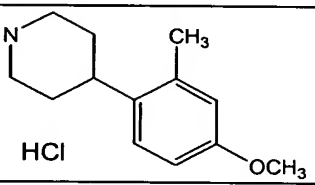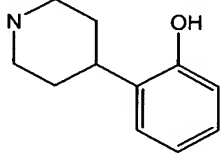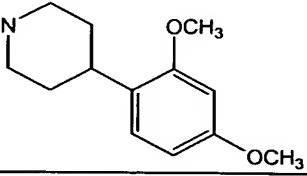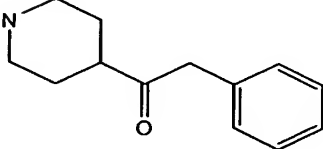
Example	R	MS (ES) m/z
278		463.1704
279		499.2304
280		
281		495.4984
282		479.1416
283		572.2800
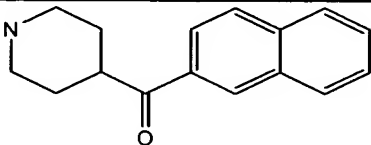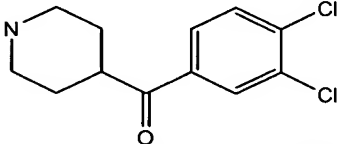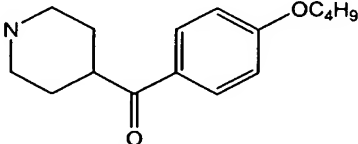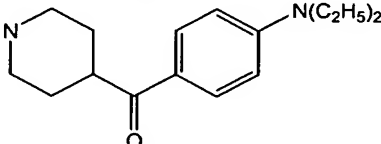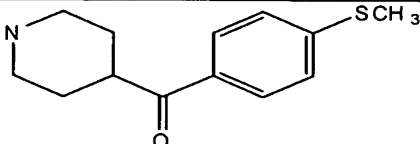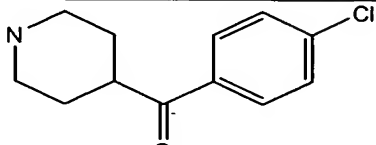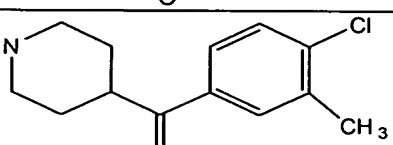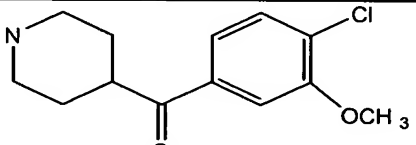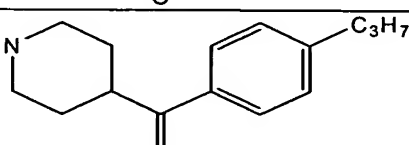
Example	R	MS (ES) m/z
284		539.2017
285		489.2049
286		477
287		515
288		483.1992
289		503
290		487
291		487
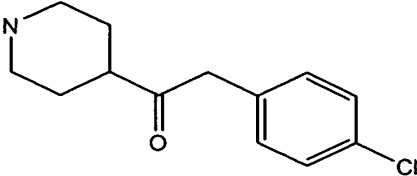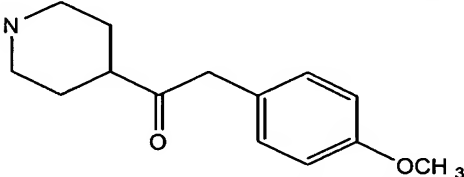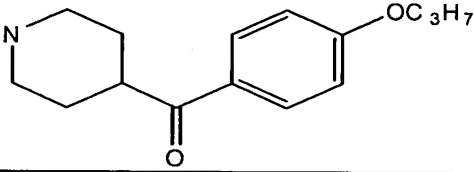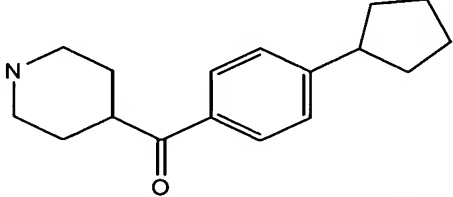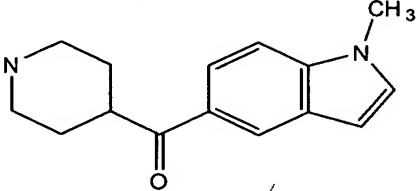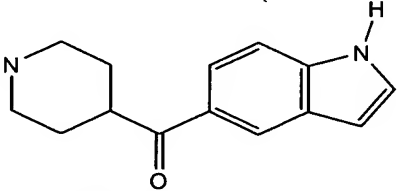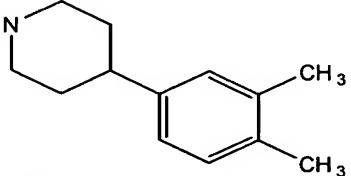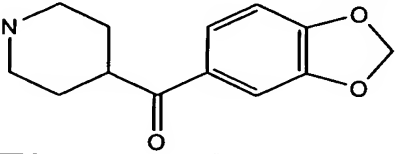
Example	R	MS (ES) m/z
292		491
293		503
294		473
295		509
296		557
297		557
298		541
299		491
300		541

129
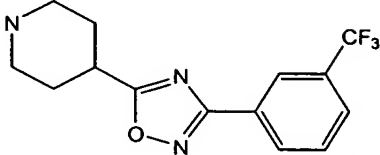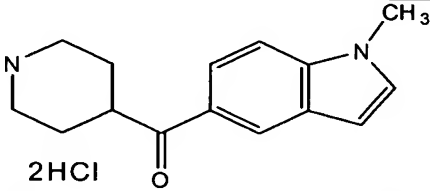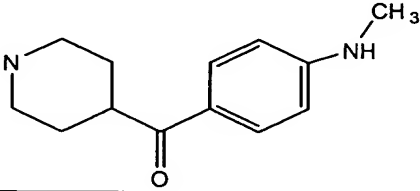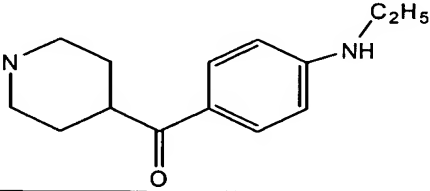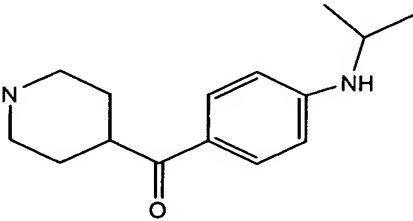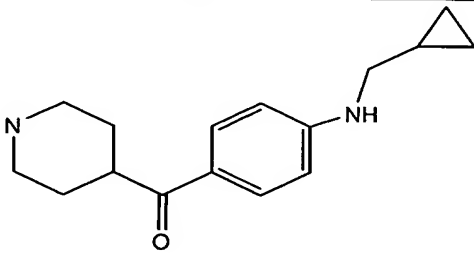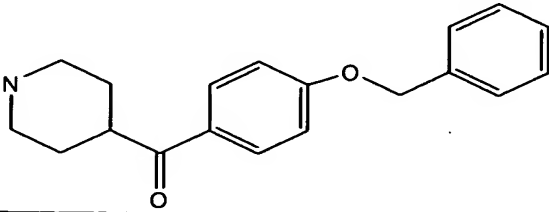
Example	R	MS (ES) m/z
301		501
302		509
303		501
304		501
305		517
306		521
307		505
308		501
309		559
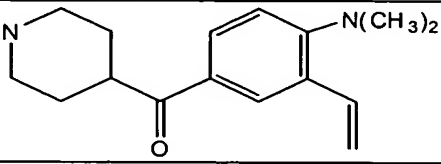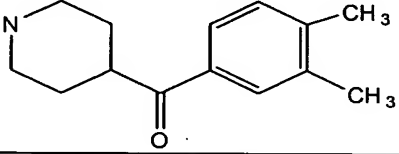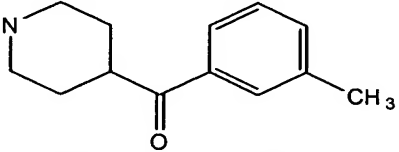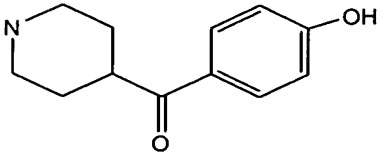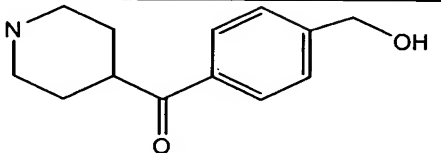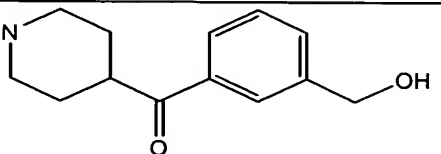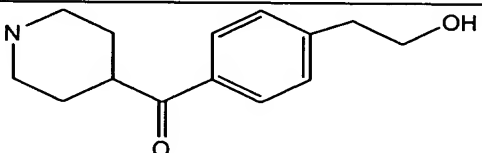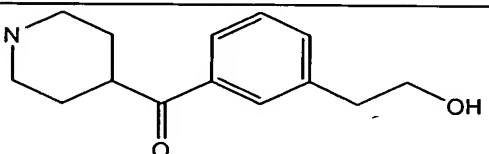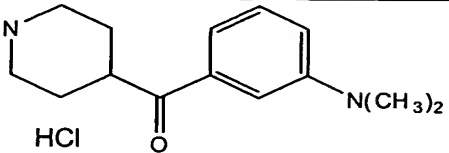
Example	R	MS (ES) m/z
310		
311		499
312		499
313		515
314		529
315		516
316		517
317		
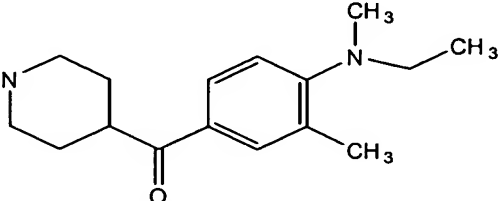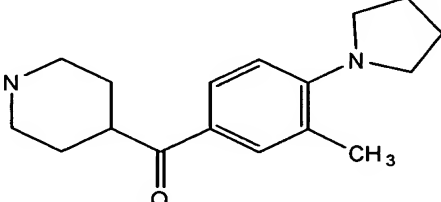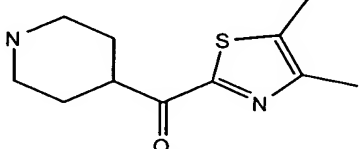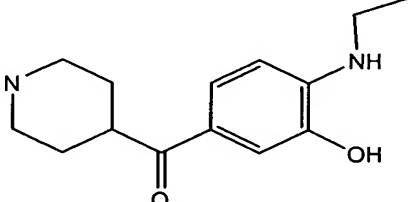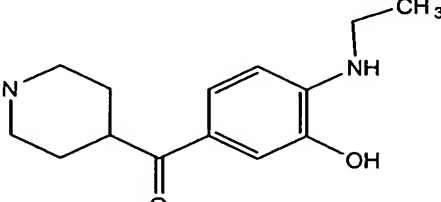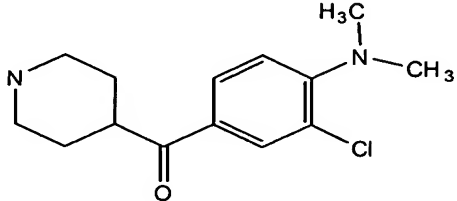
Example	R	MS (ES) m/z
318		517
319		
320		
321	HCl	
322	TFA	
323	HCl	
324		
325		
326		
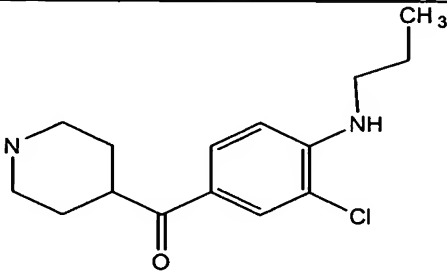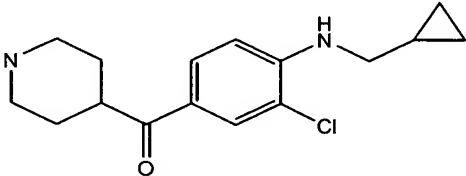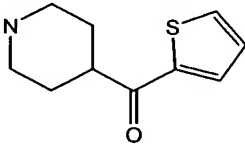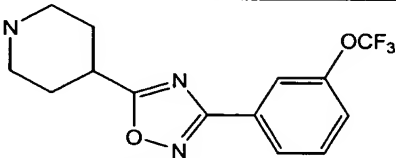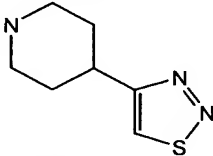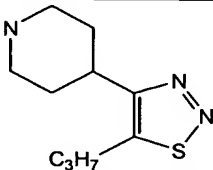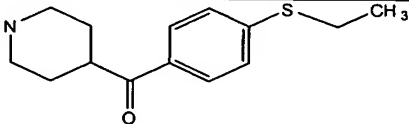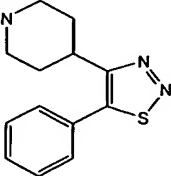
Example	R	MS (ES) m/z
327		
328		
329		
330		
331		
332		
333		
334		
335		
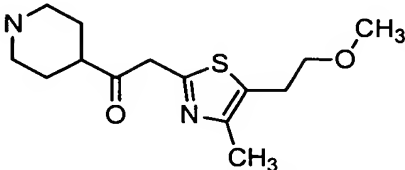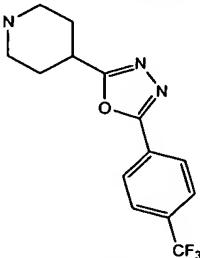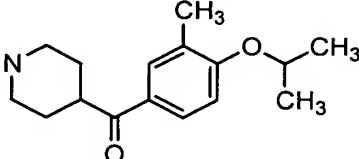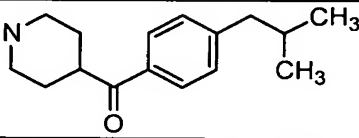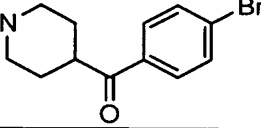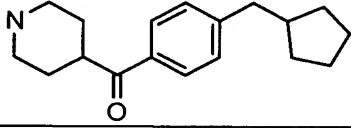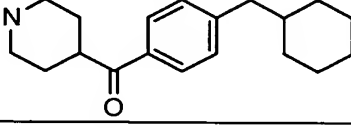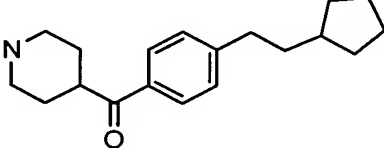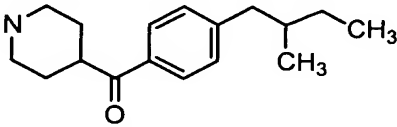
Example	R	MS (ES) m/z
336		
337		
338		
339		
340		
341		
342		
343		
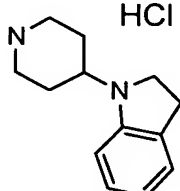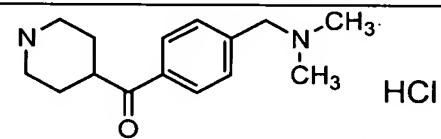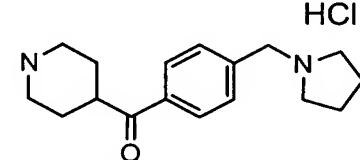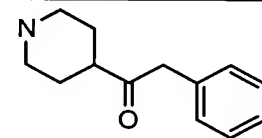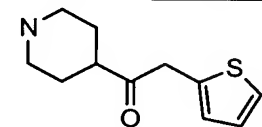
Example	R	MS (ES) m/z
344		
345	2HCl	
346		
437		
348		
349		
350		

Example	R	MS (ES) m/z
351		
352		
353		
354		
355		
356		
357		
358		
359		

Example	R	MS (ES) m/z
360		
361		
362		
363		
364		
365		

Example	R	MS (ES) m/z
366		
367		
368		
369		
370		
371		
372		
373		

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Example	R	MS (ES) m/z
374		
375		581
376		545.2320
377		529.2383
378		551.0854
379		555.252
380		569.2687
381		569.2676
382		543.2524

Example	R	MS (ES) m/z
383		
384		530.2315
385		556.2482
386		
387		

[327]   Example 388: *In Vitro* Metalloprotease Inhibition

[328]   Several hydroxamates and salts thereof were assayed for MMP

5   inhibition activity by an *in vitro* assay generally following the procedures outlined in Knight et al., *FEBS Lett.*, 296(3), 263 (1992).

[329]   Recombinant human MMP-1, MMP-2, MMP-9, MMP-13, and MMP-14 were used in this assay. These enzymes were prepared in the Assignee's laboratories following usual laboratory procedures. Specifics for preparing and

10   using these enzymes can be found in the scientific literature describing these enzymes. *See, e.g., Enzyme Nomenclature* (Academic Press, San Diego, CA, 1992) (and the citations therein). *See also*, Frije et al., *J Biol. Chem.*, 26(24), 16766-73 (1994).

[330]   The MMP-1 was obtained from MMP-1 expressing transfected
HT-1080 cells provided by Dr. Harold Welgus of Washington University in St.
Louis, MO. The MMP-1 was activated using 4-aminophenylmercuric acetate
(APMA), and then purified over a hydroxamic acid column.

5   [331]   The MMP-2 was obtained from MMP-2 expressing transfected cells
provided by Dr. Gregory Goldberg of Washington University.

[332]   The MMP-9 was obtained from MMP-9 expressing transfected
cells provided by Dr. Gregory Goldberd.

[333]   The MMP-13 was obtained as a proenzyme from a full-length
10   cDNA clone using baculovirus, as described by V.A. Luckow, "Insect Cell
Expression Technology," *Protein Engineering: Principles and Practice*, pp.
183-218 (edited by J.L. Cleland et al., Wiley-Liss, Inc., 1996). The expressed
proenzyme was first purified over a heparin agarose column, and then over a
chelating zinc chloride column. The proenzyme was then activated by APMA for
15   use in the assay. Further details on baculovirus expression systems may be found
in, for example, Luckow et al., *J. Virol.*, 67, 4566-79 (1993). *See also*, O'Reilly et
al, *Baculovirus Expression Vectors: A Laboratory Manual* (W.H. Freeman and
Co., New York, NY, 1992). *See also*, King et al., *The Baculovirus Expression
System: A Laboratory Guide* (Chapman & Hall, London, England, 1992).

20   [334]   The enzyme substrate was a methoxycoumarin-containing
polypeptide having the following sequence:

$$MCA\text{-}ProLeuGlyLeuDpaAlaArgNH_2$$

25   Here, "MCA" is methoxycoumarin and "Dpa" is
3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl alanine. This substrate is
commercially available from Baychem (Redwood City, CA) as product M-1895.

[335]   The subject hydroxamate (or salt thereof) was dissolved at various
concentrations using 1% dimethyl sulfoxide (DMSO) in a buffer containing 100

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mM Tris-HCl, 100 mM NaCl, 10 mM $CaCl_2$, and 0.05% polyethyleneglycol (23)

lauryl ether at a pH of 7.5. These solutions were then compared to a control (which

contained equal amount of DMSO/buffer solution, but no hydroxamate compound)

using Microfluor™ White Plates (Dynatech, Chantilly, VA). Specifically, The

5   MMPs were activated with APMA or trypsin. Then the various

hydroxamate/DMSO/buffer solutions were incubated in separate plates at room

temperature with the activated MMP and 4 um of the MMP substrate. The control

likewise was incubated at room temperature in separate plates with the MMP and 4

uM of the MMP substrate. In the absence of inhibitor activity, a fluorogenic

10   peptide was cleaved at the gly-leu peptide bond of the substrate, separating the

highly fluorogenic peptide from a 2,4-dinitrophenyl quencher, resulting in an

increase of fluorescent intensity (excitation at 328 nm/emission at 415). Inhibition

was measured as a reduction in fluorescent intensity as a function of inhibitor

concentration using a Perkin Elmer (Norwalk, CT) L550 plate reader. The IC_{50}'s

15   were then calculated from these measurements. The results are set forth in the

following Table A.

Inhibition Table A (nM)

Example Number	MMP-13 IC_{50}(nM)	MMP-2 IC_{50}(nM)	MMP-1 IC_{50}(nM)
4	15.6	2,900	>10000
5	15.6	2,900	>10000
6	18.1	>10000	>10000
7	18.0	4,500	>10000
8	50.0	2,500	>10000
9	12.2	5,600	>10000
10	40.0	6,000	>10000
11	37.0	2,700	>10000
12	6.70	1,400	>10000

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
13	31.6	3,500	>10000
14	45.0	>10000	>10000
15	28.0	5,500	>10000
16	42.5	4,800	>10000
17	70.0	7,000	>10000
18	>10000	>10000	>10000
19	90.0	10,000	>10000
20	23.5	4,500	>10000
21	6.00	1,600	>10000
22	10.7	3,600	>10000
23	6.40	1,600	>10000
24	6.70	700	>10000
25	4.00	445	>10000
28	10.0	800	>10000
29	20.0	4,500	>10000
30	18.1	>10000	>10000
31	15.8	2,100	>10000
32	30.0	1,750	>10000
33	67.4	6,000	67.4
34	19.3	3,700	>10000
35	26.8	900	>10000
36	70.0	5,400	>10000
37	82.5	>10000	>10000
38	17.9	5,000	>10000
39	19.0	1,050	>10000
40	80.0	5,700	>10000
41	11.4	6,000	>10000

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
42	20.0	6,500	>10000
44	40.0	5,700	>10000
45	10.0	>10000	>10000
46	20.0	2,000	>10000
47	4.10	562	>10000
48	0.2	0.3	3,000
49	2.00	59.0	>10000
50	50.0	5,000	>10000
51	2.20	0.45	>10000
52	32.6	900	>10000
53	27.8	7,000	>10000
58	28.8	900	>10000
59	110	1,000	>10000
60	11.4	1,200	>10000
70	43.5	2,050	>10000
72	80.0	10,000	>10000
73	9.00	8,300	>10000
74	76.9	10,000	>10000
75	4.80	>10000	>10000
76	32.7	2,700	>10000
77	160	>10000	>10000
78	70.0	>10000	>10000
79	37.3	>10000	>10000
80	70.0	>10000	>10000
81	19.3	>10000	>10000
82	20.0	7,300	>10000
83	90.0	>10000	>10000

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Example Number	MMP-13 IC_{50}(nM)	MMP-2 IC_{50}(nM)	MMP-1 IC_{50}(nM)
84	105	>10000	>10000
85	14.8	9,000	>10000
86	13.8	>10000	>10000
87	130	>10000	>10000
88	19.3	9,000	>10000
89	60.0	>10000	>10000
90	150	>10000	>10000
91	35.0	>10000	>10000
92	50.0	>10000	>10000
93	50.0	>10000	>10000
95	100	>10000	>10000
96	63.1	>10000	>10000
97	59.1	>10000	>1,000
98	50.0	>10000	>10000
99	50.0	>10000	>10000
100	34.9	>10000	>10000
101	40.0	>10000	>10000
102	30.6	9,000	>10000
103	37.3	>10000	>10000
104	90.0	>10000	>10000
105	175	>10000	>10000
106	115	>10000	>10000
107	30.6	7,000	>10000
108	28.6	>10000	>10000
109	60.0	>10000	>10000
110	40.0	>10000	>10000
111	40.0	10,000	>10000

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
112	48.5	>10000	>10000
113	60.0	10,000	>10000
114	120	>10000	>10000
115	200	>10000	>10000
116	77.0	>10000	>10000
117	65.0	>10000	>10000
118	420	>10000	>10000
119	1.0	200	>10000
120	0.85 (an average of 2 experiments)	126 (an average of 2 experiments)	>10000
121	0.1	58.8	>10000
122	0.1	106.5	
123	0.1	46.3	>10000
124	0.4	56.4	>10000
126	11.1	400	
127	3.0	80.0	
128	5.5	230	
129	11.4	260	
130	3.0	700	>10000
132	50.0	430	
133	1.7	16.1	>10000
134	4.5	427	>10000
135	0.5	8.0	
136	50.4	246	>10000
137	0.7	4.5	>10000
138	5.9	1500	>10000
139	1.8	330	>10000

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Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
140	18.1	800	>10000
141	1.4	160	>10000
142	6.0	420	>10000
143	2.1	100	>10000
145	210	2100	>10000
146	4.0	200	>10000
147	20.0	145	>10000
148	2.9	80.0	>10000
149	16.9	210	>10000
151	1.3	127.6	>10000
152	0.6	56.3	>10000
153	0.2	30.6	>10000
154	2.4	176.5	>10000
155	1.4	43.8	>10000
156	0.7	1335.9	>10000
157	2.7	781.6	>10000
158	2.4	217.8	>10000
159	0.5	32.2	
160	0.4	197.5	>10000
161	0.3	234.7	
162	2.7	494.6	>10000
163	3.4	3231.9	>10000
164	5.4	942.3	>10000
165	85.9	1754	
166	438	>10000	
167	4.7	2949	
168	2.1	2181.2	>10000

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
169	2.6	1061.7	>10000
170	1.3	134.1	>10000
171	1.9	405.4	>10000
172	3.1	649.1	
173	0.9	117.3	
174	1.1	1069.1	>10000
175	0.7	136.6	>10000
176	0.4	122.3	>10000
177	1.4	166.8	
178	3.0	1976.5	
179	0.7	161.3	>10000
180	0.3	52.7	>10000
181	2.3	935.7	>10000
182	1.1	115.4	>10000
183	0.7	37.9	>10000
184	1.5	360.2	>10000
185	5.1	87.4	>10000
186	3.5	94.4	>10000
187	2.7	242.4	>10000
188	2.0	249.9	>10000
189	<0.1	258	>10000
190	0.2	23.1	>10000
191	3.0	2286.9	>10000
192	1.3	103.3	>10000
193	0.4	98.7	>10000
194	9.1	1229.7	>10000
195	0.3	462.8	>10000

Example Number	MMP-13 IC$_{50}$(nM)	MMP-2 IC$_{50}$(nM)	MMP-1 IC$_{50}$(nM)
196	1.0	750.1	>10000
197	1.4	1720.1	>10000
198	12.0	2565.6	
199	11.7	3390.0	>10000
200	0.5	1398.8	>10000
201	0.2	6315.4	>10000
202	0.4	1017.6	>10000
203	0.6	816.4	2367
204	0.2	1045.8	>10000
205	<0.1	411.5	>10000
206	1.8	199.4	>10000
207	1.1	4.4	>10000
208	0.1	19.6	>10000
210	1.1	13.1	>10000
211	1.2	122.3	>10000
212	0.2	109.7	>10000
213	0.5	25.8	>10000
214	1.7	159.8	>10000
215	0.9	22.7	>10000
216	1.5	46.4	>10000
217	1.3	270.0	>10000
218	0.2	75.7	>10000
219	4.9	258.2	>10000
220	1.7	289.8	>10000
221	3.4	301.1	>10000
222	1.0	196.6	>10000
223	2.5	80.4	>10000

Example Number	MMP-13 IC$_{50}$(nM)	MMP-2 IC$_{50}$(nM)	MMP-1 IC$_{50}$(nM)
224	0.4	72.9	>10000
225	0.2	40.8	>10000
226	<0.1	1024	>10000
227	1.4	132.1	>10000
228	19.5	154.6	
229	0.2	8.5	>10000
230	0.1	745.0	>10000
231	0.5	39.4	>10000
232	1.3	624.4	>10000
233	1.2	1046.1	>10000
234	7.5	2444.7	>10000
235	0.8	118.0	>10000
236	1.5	1848.4	>10000
237	2.1	1914.8	>10000
238	1.8	62.1	
239	0.6	75.8	
240	2.8	86.0	
242	1.0	87.5	
243	0.3	56.0	>10000
244	0.2	15.2	
245	1.1	38.6	
246	1.0	2712.9	>10000
247	0.3	111.4	>10000
248	0.6	141.0	>10000
249	5.8	>10000	>10000
250	2.1	107.2	
252	0.4	14.3	

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
253	1.7	38.7	>10000
254	1.3	132.0	>10000
256	7.5	35.4	
257			
258	14.1	45.4	
259	0.4	0.6	
260	0.4	1.2	>10000
261	0.8	1.0	
262	1.0	1.7	
263	1.5	2.6	>10000
264	0.8	3.1	
265	0.5	3.2	
266	1.7	4.5	
267	0.4	1.7	>10000
268	1.2	5.0	>10000
269	1.4	4.5	>10000
270	1.1	1.9	>10000
271	0.8	1.7	>10000
272	1.3	5.9	>10000
273	2.5	13.4	
274	2.1	5.2	>10000
275	183.6	6736.9	
276	126.7	2733.4	
277	274.5	>10000	
279	160	3300	>10000
280	27.1	500	>10000
281	11.4	500	>10000

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
282	0.7	2.0	>10000
284	33.7	5400	>10000
285	35.0	3100	>10000
287	70.0	>10000	>10000
288	4.4	60.7	>10000
289	6.0	160	>10000
290	0.4	82.0	>10000
291	0.8	160	>10000
292	3.2	35.0	>10000
293	37.3	1400	>10000
294	3.1	120	>10000
295	28.6	300	>10000
296	25.1	210	>10000
297	15.8	250	>10000
298	34.9	240	>10000
299	9.4	106	>10000
300	14.8	240	>10000
301	37	3000	>10000
302	1.9	35	>10000
303	3.1	590	>10000
304	1.6	270	>10000
305	6.0	3300	>10000
306	9.0	800	>10000
307	0.9	145	>10000
308	3.0	1280	>10000
309	22.0	270	>10000
310	6.0	4500	>10000

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
311	3.7	700	>10000
312	1.2	175	>10000
313	3.0	445	>10000
314	12.2	3700	>10000
315	4.5	700	>10000
316	2.0	700	>10000
317	4.0	23.5	>10000
318	5.7	130	>10000
319	4.0	175	>10000
320	2.3	10,000	>10000
321	200	1400	>10000
322	140	1400	>10000
323	7.0	505	>10000
324	11.3	70.0	>10000
325	11.0	1750	>10000
326	3.0	70.0	>10000
327	5.0	4700	>10000
328	4.5	186	>10000
329	20.0	1800	ND
330	--	--	ND
331	1.2	250	ND
332	1.3	120	ND
333	3.7	600	>10000
334	5.5	440	ND
335	2.7	1500	>10000
336	2.0	34.9	ND
337	1.7	40.0	ND

Example Number	MMP-13 $IC_{50}(nM)$	MMP-2 $IC_{50}(nM)$	MMP-1 $IC_{50}(nM)$
338	--	--	ND
339	--	--	ND
340	16.5	10,000	>10000
341	--	--	ND
342	2.0	76.9	ND
374	5.6	970	>10000
375	34.4	2663	
376	6.4	2185.4	>10000
377	0.4	361.4	>10000
378	0.3	28.4	>10000
379	0.6	1266.4	>10000
380	9.7	2287.6	>10000
381	3.5	639.9	>10000
382	0.3	1305.4	>10000
383	36.9	382.4	
384	2.9	52.9	
385	3.2	34.6	
386	15.2	1901.1	
387	4.5	344.4	

[336]   Example 389: *In Vivo* Angiogenesis Assay

[337]   The study of angiogenesis depends on a reliable and reproducible model for the stimulation and inhibition of a neovascular response. The corneal micropocket assay provides such a model of angiogenesis in the cornea of a mouse. *See, A Model of Angiogenesis in the Mouse Cornea*; Kenyon,BM, et al., Investigative Ophthalmology & Visual Science, July 1996, Vol. 37, No. 8.

[338] In this assay, uniformLy sized Hydron™ pellets containing bFGF and sucralfate were prepared and surgically implanted into the stroma mouse cornea adjacent to the temporal limbus. The pellets were formed by making a suspension of 20 μL sterile saline containing 10 μg recombinant bFGF, 10 mg of sucralfate and 10 μL of 12 percent Hydron™ in ethanol. The slurry was then deposited on a 10 x 10 mm piece of sterile nylon mesh. After drying, the nylon fibers of the mesh were separated to release the pellets.

[339] The corneal pocket is made by anesthetizing a 7 week old C57Bl/6 female mouse, then proptosing the eye with a jeweler's forceps. Using a dissecting microscope, a central, intrastromal linear keratotomy of approximately 0.6 mm in length is performed with a #15 surgical blade, parallel to the insertion of the lateral rectus muscle. Using a modified cataract knife, a lamellar micropocket is dissected toward the temporal limbus. The pocket is extended to within 1.0 mm of the temporal limbus. A single pellet was placed on the corneal surface at the base of the pocket with a jeweler's forceps. The pellet was then advanced to the temporal end of the pocket. Antibiotic ointment was then applied to the eye.

[340] Mice were dosed on a daily basis for the duration of the assay. Dosing of the animals was based on bioavailability and overall potency of the compound. an exemplary dose was 10 or 50 mg/kg (mpk) bid, po. Neovascularization of the corneal stroma begins at about day three and was permitted to continue under the influence of the assayed compound until day five. At day five, the degree of angiogenic inhibition was scored by viewing the neovascular progression with a slit lamp microscope.

[341] The mice were anesthetized and the studied eye was once again proptosed. The maximum vessel length of neovascularization, extending from the limbal vascular plexus toward the pellet was measured. In addition, the contiguous circumferential zone of neovascularization was measured as clock hours, where 30 degrees of arc equals one clock hour. The area of angiogenesis was calculated as follows.

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$$\text{area} = \frac{(0.4 \text{xclock hours} \times 3.14 \times \text{vessel length (in mm)})}{2}$$

[342] Five to six mice were utilized for each compound in each study. The studied mice were thereafter compared to control mice and the difference in the area of neovascularization was recorded as an averaged value. Each group of mice so studied constitutes an "n" value of one, so that "n" values greater than one represent multiple studies whose averaged result is provided in the table. A contemplated compound typically exhibits about 25 to about 75 percent inhibition, whereas the vehicle control exhibits zero percent inhibition.

[343] **Example 390: *In Vivo* PC-3 Tumor Reduction**

[344] PC-3 human pancreatic cancer eclls (ATCC CRL 1435) were grown to 90% confluence in F12/MEM (Gibco) containing 7% FBS (Gibco). Cells were mechanically harvested using a rubber scraper, and then washed twice with cold medium. The resulting cells were resuspended in cold medium with 30% matrigel (Collaborative Research) and the cell-containing medium was maintained on ice until used.

[345] Balb/c nu/nu mice at 7-9 weeks of age were anesthetized with avertin [2,2,2-tribromethanol/t-amyl alcohol (1 g/1 mL) diluted 1:60 into phosphate-buffered sline] and $3\text{-}5\text{x}10^6$ of the above cells in 0.2 mL of medium were injected into the left flank of each mouse. Cells were injected in the morning, whereas dosing with an inhibitor began at 6 PM. The animals were gavaged BID from day zero (cell injection day) to day 25-30, at which time the animals were euthanized and tumors weighed.

[346] Compounds were dosed at 10 mg/mL in 0.5% methylcellulose/0.1% polysorbate 80 to provide a 50 mg/kg (mpk) dose twice each day, or diluted to provide a 10 mg/kg (mpk) dose twice each day. Tumor measurements began on day 7 and continued every third or fourth day until completion of the study.

Groups of ten mice were used in each study and nine to ten survived. Each group of mice so studied constitutes an "n" value of one, so that "n" values greater than one represent multiple studies whose averaged result is provided in the table.

5   **[347]   Example 391: Tumor Necrosis Factor Assays**

[348] Cell Culture.

[349] The cells used in the assay are the human moncytic line U-937 (ATCC CRL-1593). The cells are grown in RPMI w/10% FCS and PSG supplement (R-10) and are not permitted to overgrow. The assay is carried out as

10   follows:

[350] 1. Count, then harvest cells by centrifugation. Resuspend the pellet in R-10 supplement to a concentration of 1.540×10^6 cells/mL.

[351] 2. Add test compound in 65 uL R-10 to the appropriate wells of a 96-well flat bottom tissue culture plate. The initial dilution from a DMSO stock

15   (100 mM compound) provides a 400 uM solution, from which five additional three-fold serial dilutions are made. Each dilution of 65 ul (in triplicate) yields final compound test concentrations of 100 μM, 33.3 μM, 11.1 μM, 3.7 μM, 1.2 μM and 0.4 μM.

[352] 3. The counted, washed and resuspended cells (200,000 cells/well)

20   in 130 μL are added to the wells.

[353] 4. Incubation is for 45 min to 1 hr at 37°C in 5% CO_2 in a water saturated container.

[354] 5. R-10 (65 uL)containing 160 ng/mL PMA (Sigma) is added to each well.

25   **[355]** 6. The test system is incubated at 37°C in 5% CO_2 overnight (18-20 hr) under 100% humidity.

[356] 7. Supernatant, 150 μL, is carefully removed from each well for use in the ELISA assay.

[357]   8. For toxicity, a 50 µL aliquot of working solution containg 5 mL R-10, 5 mL MTS solution [CellTiter 96 AQueous One Solution Cell Proliferation Assay Cat.#G358/0,1 (Promega Biotech)] and 250 ul PMS solution are added to each well containing the remaining supernatant and cells and the cells incubated at 37°C in 5% CO_2 until the color develops. The system is excited at 570 nm and read at 630 nm.

[358]   TNF Receptor II ELISA Assay

[359]   1. Plate 100 µL/well 2 ug/mL mouse anti-human TNFrII antibody (R&D Systems #MAB226) in 1 x PBS (pH 7.1, Gibco) on NUNC-Immuno Maxisorb plate. Incubate the plate at 4°C overnight (about 18-20 hr).

[360]   2. Wash the plate with PBS-Tween (1 x PBS w/ 0.05% Tween).

[361]   3. Add 200 µL 5% BSA in PBS and block at 37°C in a water saturated atmosphere for 2 hr.

[362]   4. Wash the plate with PBS-Tween.

[363]   5. Add sample and controls (100 ul of each) to each well. The standards are 0, 50, 100, 200, 300 and 500 pg recombinant human TNFrII (R&D Systems #226-B2) in 100 µL 0.5% BSA in PBS. The assay is linear to between 400-500 pg of standard.

[364]   6. Incubate at 37°C in a saturated atmosphere for 1.5 hr.

[365]   7. Wash the plate with PBS-Tween.

[366]   8. Add 100 µL goat anti-human TNFrII polyclonal (1.5 µg/mL R&D Systems #AB226-PB in 0.5% BSA in PBS).

[367]   Incubate at 37°C in a saturated atmosphere for 1 hr.

[368]   10. Wash the plate with PBS-Tween.

[369]   11. Add 100 µL anti-goat IgG-peroxidase (1:50,000 in 0.5% BSA in PBS, Sigma #A5420).

[370]   11. Incubate at 37°C in a saturated atmosphere for 1 hr.

[371]   12. Wash the plate with PBS-Tween.

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[372] 13. Add 10 μL KPL TMB developer, develop at room temperature (usually about 10 min), then terminate with phosphoric acid and excite at 450 nm and read at 570 nm.

5   [373] TNFα ELISA Assay.

[374] Coat Immulon® 2 plates with 0.1 mL/well of 1ug/mL Genzyme mAb in 0.1 M NaHCO3 pH 8.0 buffer overnight (about 18-20 hr) at 4°C, wrapped tightly in Saran® wrap.

[375] Flick out coating solution and block plates with 0.3 mL/well

10   blocking buffer overnight at 4°C, wrapped in Saran® wrap.

[376] Wash wells thoroughly 4X with wash buffer and completely remove all wash buffer. Add 0.1 mL/well of either samples or rhTNFα standards. Dilute samples if necessary in appropriate diluant (e.g. tissue culture medium). Dilute standard in same diluant. Standards and samples should be in triplicates.

15   [377] Incubate at 37°C for 1 hr in humified container.

[378] Wash plates as above. Add 0.1 mL/well of 1:200 dilution of Genzyme rabbit anti-hTNFa.

[379] Repeat incubation.

[380] Repeat wash. Add 0.1 mL/well of 1 μg/mL Jackson goat anti-rabbit

20   IgG (H+L)-peroxidase.

[381] Incubate at 37°C for 30 min.

[382] Repeat wash. Add 0.1 mL/well of peroxide-ABTS solution.

[383] Incubate at room temperature for 5-20 min.

[384] Read OD at 405 nm.

25

[385] 12 Reagents are:

Genzyme mouse anti-human TNF? monoclonal (Cat.# 80-3399-01)

Genzyme rabbit anti-human TNF? polyclonal (Cat.#IP-300)

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Genzyme recombinant human TNF? (Cat.#TNF-H).

Jackson Immunoresearch peroxide-conjugated goat anti-rabbit IgG (H+L) (Cat.#111-035-144).

Kirkegaard/Perry peroxide ABTS solution (Cat#50-66-01).

5 Immulon 2 96-well microtiter plates.

Blocking solution is 1 mg/mL gelatin in PBS with 1X thimerasol.

Wash buffer is 0.5 mL Tween® 20 in 1 liter ofPBS.

[386] Example 392: *In Vitro* Aggrecanase Inhibition Assay

10   [387] Assays for measuring the potency (IC_{50}) of a compound toward inhibiting aggrecanase are known in the art.

[388] One such assay, for example, has been reported in European Patent Application Publ. No. EP 1 081 137 A1. In that assay, primary porcine chondrocytes from articular joint cartilage are isolated by sequential trypsin and

15 collagenase digestion followed by collagenase digestion overnight and are plated at 2×10^5 cells per well into 48 well plates with 5 $\mu Ci/ml^{35}S$ (1000 Ci/mmol) sulphur in type 1 collagen coated plates. Cells are allowed to incorporate label into their proteoglycan matrix (approximately 1 week) at 37°C under an atmosphere of 5% CO_2. The night before initiating the assay, chondrocyte monolayers are washed 2

20 times in DMEM/1% PSF/G and then allowed to incubate in fresh DMEM/1% FBS overnight. The next morning, chondrocytes are washed once in DMEM/1% PSF/G. The final wash is allowed to sit on the plates in the incubator while making dilutions. Media and dilutions are made as described in the following Table C:

25   Table C

control media	DMEM alone
IL-1 media	DMEM + IL-1 (5ng/ml)

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drug dilutions	Make all compound stocks at 10 mM in DMSO.
.	Make a 100 μM stock of each compound in DMEM in 96-well plate. Store in freezer overnight.
	The next day, perform serial dilutions in DMEM with IL-1 to 5 μM, 500 nM, and 50 nM.
	Aspirate final wash from wells and add 50 μM of compound from above dilutions to 450 μL of IL-1 media in appropriate wells of the 48 well plates.
	Final compound concentrations equal 500 nM, 50 nM, and 5 nM. All samples completed in triplicate with control and IL-1 alone on each plate.

Plates are labeled and only the interior 24 wells of the plate are used. On one of the plates, several columns are designated as IL-1 (no drug) and control (no IL-1, no drug). These control columns are periodically counted to monitor 35S-

5   proteoglycan release. Control and IL-1 media are added to wells (450 μL) followed by compound (50 μL) so as to initiate the assay. Plates are incubated at 37°C with 5% CO_2 atmosphere. At 40-50% release (when CPM from IL-1 media is 4-5 times control media) as assessed by liquid scintillation counting (LSC) of media samples, the assay is terminated (about 9 to about 12 hours). Media is removed from all

10 wells and placed into scintillation tubes. Scintillate is added and radioactive counts are acquired (LSC). To solubilize cell layers, 500 μL of papain digestion buffer (0.2 M Tris, pH 7.0, 5 mM DTT, and 1 mg/ml papain) is added to each well. Plates with digestion solution are incubated at 60°C overnight. The cell layer is removed from the plates the next day and placed in scintillation tubes. Scintillate

15 is then added, and samples counted (LSC). The percent of released counts from the total present in each well is determined. Averages of the triplicates are made with control background subtracted from each well. The percent of compound inhibition is based on IL-1 samples as 0% inhibition (100% of total counts).

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[389] Another assay for measuring aggrecanase inhibition has been reported in WIPO Int'l Publ. No. WO 00/59874. That assay reportedly uses active aggrecanase accumulated in media from stimulated bovine cartilage (BNC) or related cartilage sources and purified cartilage aggrecan monomer or a fragment

5   thereof as a substrate. Aggrecanase is generated by stimulation of cartilage slices with interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), or other stimuli. To accumulate BNC aggrecanase in culture media, cartilage reportedly is first depleted of endogenous aggrecan by stimulation with 500 ng/ml human recombinant IL-β for 6 days with media changes every 2 days. Cartilage is then stimulated for an

10   additional 8 days without media change to allow accumulation of soluble, active aggrecanase in the culture media. To decrease the amounts of matrix metalloproteinases released into the media during aggrecanase accumulation, agents which inhibit MMP-1, -2, -3, and -9 biosynthesis are included during stimulation. This BNC conditioned media containing aggrecanase activity is then

15   used as the source of aggrecanase for the assay. Aggrecanase enzymatic activity is detected by monitoring production of aggrecan fragments produced exclusively by cleavage at the Glu373-Ala374 bond within the aggrecan core protein by Western analysis using the monoclonal antibody, BC-3 (Hughes, et al., *Biochem J*, 306:799-804 (1995)). This antibody reportedly recognizes aggrecan fragments with the N-

20   terminus, 374ARGSVIL, generated upon cleavage by aggrecanase. The BC-3 antibody reportedly recognizes this neoepitope only when it is at the N-terminus and not when it is present internally within aggrecan fragments or within the aggrecan protein core. Only products produced upon cleavage by aggrecanase reportedly are detected. Kinetic studies using this assay reportedly yield a Km of

25   1.5+/-0.35 μM for aggrecanase. To evaluate inhibition of aggrecanase, compounds are prepared as 10 mM stocks in DMSO, water, or other solvents and diluted to appropriate concentrations in water. Drug (50 μL) is added to 50 μL of aggrecanase-containing media and 50 μL of 2 mg/ml aggrecan substrate and brought to a final volume of 200 μL in 0.2 M Tris, pH 7.6, containing 0.4 M NaCl

and 40 mM $CaCl_2$. The assay is run for 4 hr at 37°C, quenched with 20 mM EDTA, and analyzed for aggrecanase-generated products. A sample containing enzyme and substrate without drug is included as a positive control and enzyme incubated in the absence of substrate serves as a measure of background. Removal of the

5   glycosaminoglycan side chains from aggrecan reportedly is necessary for the BC-3 antibody to recognize the ARGSVIL epitope on the core protein. Therefore, for analysis of aggrecan fragments generated by cleavage at the Glu373-Ala374 site, proteoglycans and proteoglycan fragments are enzymatically deglycosylated with chondroitinase ABC (0.1 units/10 μg GAG) for 2 hr at 37°C and then with

10   keratanase (0.1 units/10 μg GAG) and keratanase II (0.002 units/10 μg GAG) for 2 hr at 37°C in buffer containing 50 mM sodium acetate, 0.1 M Tris/HCl, pH 6.5. After digestion, aggrecan in the samples is precipitated with 5 volumes of acetone and resuspended in 30 μL of Tris glycine SDS sample buffer (Novex) containing 2.5% beta mercaptoethanol. Samples are loaded and then separated by SDS-PAGE

15   under reducing conditions with 4-12% gradient gels, transferred to nitrocellulose and immunolocated with 1:500 dilution of antibody BC3. Subsequently, membranes are incubated with a 1:5000 dilution of goat anti-mouse IgG alkaline phosphatase second antibody and aggrecan catabolites visualized by incubation with appropriate substrate for 10-30 minutes to achieve optimal color development.

20   Blots are quantitated by scanning densitometry and inhibition of aggrecanase determined by comparing the amount of product produced in the presence versus absence of compound.

* * * * * * * * *

25   [390] The above detailed description of preferred embodiments is intended only to acquaint others skilled in the art with the invention, its principles, and its practical application so that others skilled in the art may adapt and apply the invention in its numerous forms, as they may be best suited to the requirements of a

particular use. This invention, therefore, is not limited to the above embodiments, and may be variously modified.